Guidelines for Sampling Freshwater Mussels in Wadable Streams

Wisconsin Department of Natural Resources,
Fisheries and Aquatic Sciences Research Program

Final Report No. 0092-01-09
March 2005

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Notice

This research was funded by the Wisconsin Council on Research of the Wisconsin Department of Transportation and the Federal Highway Administration under Project #SPR-0092-01-09. The contents of this report reflect the views of the authors who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views of the Wisconsin Department of Transportation or the Federal Highway Administration at the time of publication.

Technical Report Documentation Page

1. Report No. 0092-01-09	2. Government Accession No	3. Recipient's Catalog No
4. Title and Subtitle Guidelines For Sampling Freshwater Mussels In Wadable Streams		5. Report Date March 2005 6. Performing Organization Code
7. Authors Randal R. Piette		8. Performing Organization Report No. 0092-01-09
9. Performing Organization Name and Address Wisconsin Department of Natural Resources Fisheries and Aquatic Sciences Research Program		10. Work Unit No. (TRAIS) 11. Contract or Grant No.
12. Sponsoring Agency Name and Address Wisconsin Department of Transportation, Council on Research 4802 Sheboygan Ave., Room 451 P.O. Box 7965 Madison, WI 53707-7965		13. Type of Report and Period Covered Final Report 14. Sponsoring Agency Code
15. Supplementary Notes		

16. Abstract

Highway construction activities, especially bridge projects, can disturb the habitat of mussels and other aquatic life. To preserve these creatures and comply with state and federal regulations, the Wisconsin Department of Transportation surveys streams for mussels before construction begins. Because of the lack of statewide mussel population data prior to this project, most bridge construction projects required a costly and time-consuming underwater study.

Funded as a joint project between WisDOT and the Wisconsin Department of Natural Resources, this study provides a comprehensive baseline inventory of mussels in Wisconsin and a prioritized listing of geographic areas and waterbodies for future mussel survey work. The GIS-based Wisconsin Mussel Atlas produced in this project will allow WisDOT to quickly locate sites that have previously been inventoried, which may mean a new survey is not required before bridge construction. Eliminating a mussel survey can save considerable time and money.

This project also developed standard mussel sampling and reporting protocols for wadable bodies of water. These guidelines will standardize the collection of mussel data, reducing collector bias and sampling error and facilitating statewide comparisons of mussel communities and habitat.

17. Key Words Freshwater Mussels Survey Protocols	18. Distribution Statement No restriction. This document is available to the public through the National Technical Information Service 5285 Port Royal Road Springfield VA 22161		
18. Security Classif.(of this report) Unclassified	19. Security Classif. (of this page) Unclassified	20. No. of Pages	21. Price

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

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Executive Summary

Freshwater mussels fill a variety of ecological roles and are essential to maintaining the integrity of aquatic ecosystems. They help maintain water quality through their filter feeding, provide a stable forage base for fish, furbearers, and other wildlife species, are potentially useful environmental indicators, and have commercial value. Unfortunately, freshwater mussels also comprise one of the most endangered faunas in the United States. Of all groups of organisms tracked by state Natural Heritage Inventory program, unionid mussels have the highest percentage of individual species that are tracked. Currently, 18 mussel species are listed as endangered or threatened by the state of Wisconsin.

Highway construction activities, especially bridge projects, can disturb the habitat of mussels and other aquatic life. To preserve these creatures and comply with state and federal regulations, the Wisconsin Department of Transportation surveys streams for mussels before bridge construction begins, and may need to relocate any mussels found. In the past, methods of collecting mussels and mussel habitat information have been left to the discretion of individual biologists. Methods differ and include simple random searches of varying length, time and effort. Because of this, there is little quantitative data available for comparisons of mussel populations across the state. In addition, each contractor that performed mussel surveys and relocation for WisDOT had to develop collection procedures before beginning work, which increased the time and money required for the project.

This study serves as a first step in addressing this lack of mussel data and the inconsistencies in survey methods. Funded as a joint project between the Wisconsin Department of Transportation and the Wisconsin Department of Natural Resources, it provides a comprehensive baseline inventory of mussels in Wisconsin, standardized mussel sampling and reporting protocols for wadable rivers and streams and wadable portions of large rivers, and a prioritized listing of geographic areas/waterbodies for future mussel survey work.

Wisconsin Mussel Atlas

A key component of this research was the development of an electronic atlas, which provides a baseline inventory of mussels in Wisconsin. The Atlas includes details from historical mussel survey sites, including published and unpublished (but verified) records of individual mussel occurrences. The Atlas data will serve as a foundation for further mussel inventories in the future.

Priority Watersheds

Information developed as part of the electronic atlas compilation has been used to identify significant gaps in the mussel distribution data. Gaps identified include areas that have not previously been surveyed, areas where only limited (usually qualitative) surveys have been completed, and areas for which only very old (> 30 years old) records are available. These sites have been prioritized for future survey efforts.

Solicitation of priority waters for future mussel surveys resulted in a long list of waters, with none of the waters being nominated more than once. This strongly indicates the great need for more mussel work to be conducted on many of the state's waterways. This report includes a listing of 15 waterbodies that should be considered for mussel sampling.

Sampling Protocols

The bulk of this report consists of standardized sampling and reporting protocols to survey and assess freshwater mussel populations in wadable streams and rivers in conjunction with current WDNR basin surveys. These protocols represent a starting point for conducting mussel surveys to provide point-in-time as well as baseline data for evaluating and monitoring mussel communities in wadable streams.

Development of the protocols was based on review of existing literature and our own research and experiences. Emphasis was placed on providing reliable and repeatable survey techniques that balanced field efforts and the collection of meaningful biological data. The protocols by no means represent the final word on mussel and mussel habitat evaluation, however the purpose is to begin to standardize mussel and mussel habitat surveys conducted in Wisconsin.

The mussel sampling protocols established within this document are designed to answer three primary objectives associated with sampling mussel communities in wadable streams: 1) Are mussels present; 2) Which species are present; and 3) What is the density of mussels and habitat relationship. Three different sampling protocols were developed that can be used to address the primary objectives.

Development of freshwater mussel sampling protocols for non-wadable waters was not done at this time due to the need to use SCUBA equipment and lack of trained personnel. Many of the procedures developed for wadable waters (station location, qualitative sampling) can likely be modified for sampling non-wadable waters, however, quantitative sampling techniques will need to be developed, tested and refined. Establishing standardized sampling protocols for non-wadable waters should be considered a high priority for mussel management in the state.

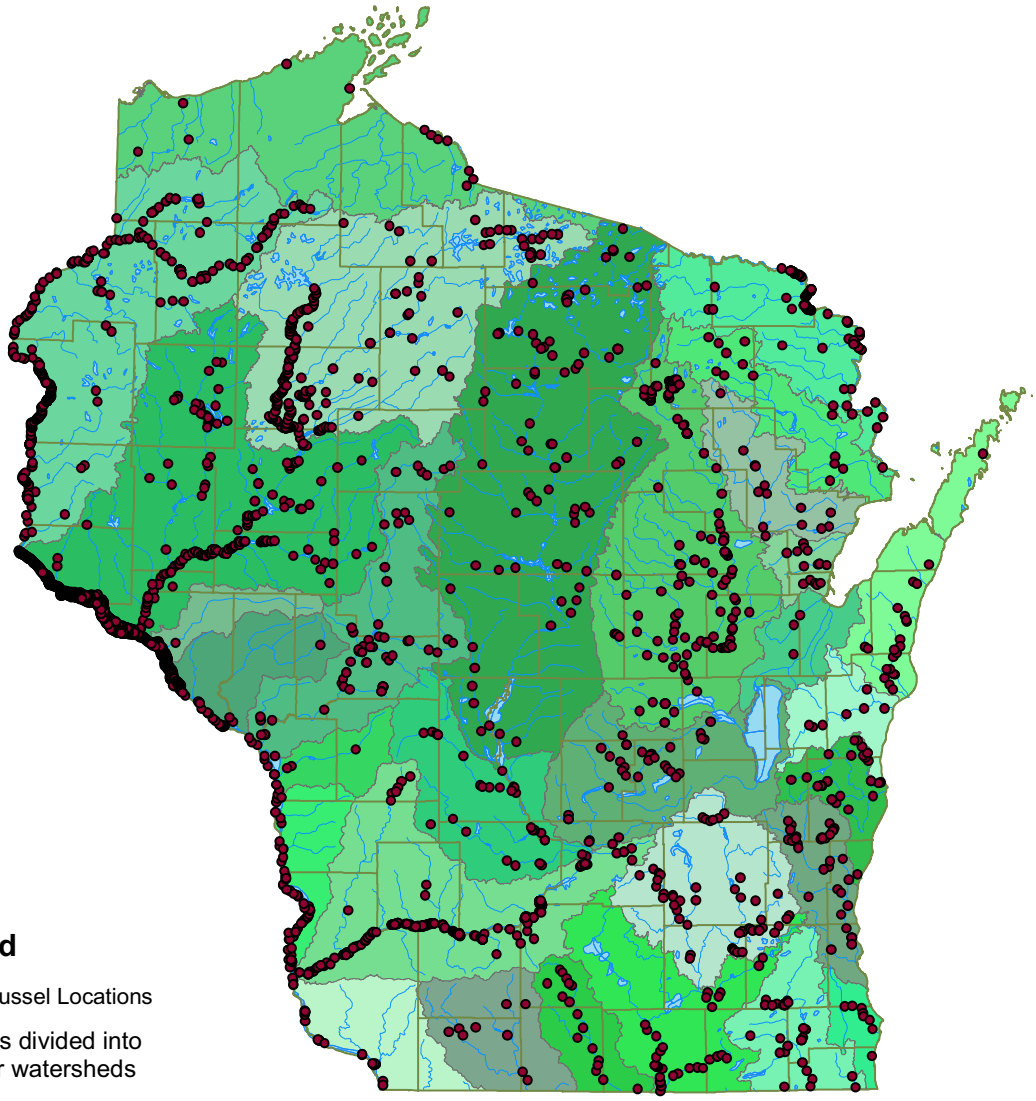
Wisconsin Mussel Atlas

This research included documentation of the known, historical distribution of Wisconsin's unionid mussel resources. By capturing this known inventory in an electronic database, we're laying the necessary groundwork for a complete Wisconsin Mussel Atlas, which would contain the results of current mussel surveys across the state. On page four is an image of the initial GIS map of known mussel sites that are stored in this database. Some of the information that is part of this initial electronic Atlas database includes historical mussel survey sites, approximate site locations separated by water basin and river, collector, date collected, species collected, and number of living and dead shells found. The electronic atlas includes published and unpublished (but verified) records of individual mussel occurrences.

The bulk of these points originate from the 1970's with a few even earlier (e.g., a few references in the southeastern counties originated from the 1930's.) Each site was located by using author's descriptions and is approximated on this map. Location was especially troublesome in the large Mississippi pools where some authors would only mention pool numbers for locations.

This data can be used for several purposes, including gap analysis for both current and historical mussel ranges for Wisconsin, and historical and current water quality indicators. The data can also be used as a foundation for further mussel inventories in the future. Its distribution will include universities and natural resource agencies of the upper mid-west so that it could be joined with data from other states to present an even wider picture of mussel distribution and densities for the entire upper mid-west.

Wisconsin Mussel Atlas



Legend

- Mussel Locations

State is divided into major watersheds

A cooperative effort between the Department of Natural Resources and the Department of Transportation

Priority Watersheds

Solicitation of priority waters for future mussel surveys resulted in a long list of waters, with none of the waters being nominated more than once. This strongly indicates the great need for more mussel work to be conducted on many of the state's waterways. Many of the tributaries to the Mississippi have had their lower reaches surveyed for mussels, however, much of their headwaters remain to be surveyed. North-central and Northeastern portions of the state have had relatively few mussel surveys, along with south-central areas. Waters in northern portions of the state remain in relatively good conditions and do not suffer as much from land-use problems as southern portions of the state. Nearly all of the larger waters suffer from fragmentation caused by dams, which block host fish migrations thereby limiting mussel distribution.

A top priority for monitoring the mussel communities within the state should be the development of a long-term monitoring program that establishes quantitative baseline information at approximately 30 sites distributed across the state. These sites could then be monitored on a 5 – 10 year basis to determine long-term trends in the mussel community. Standardized quantitative sampling should be conducted at any site where planned disturbances may affect the mussel community and it is necessary to determine the impact. Qualitative surveys need to follow a standardized sampling protocol, and should be used primarily for species distribution.

In terms of priority waters or watersheds, the following waterbodies should be considered for mussel sampling:

1) Tribs of the Illinois-Fox in Wisconsin (Racine and Walworth Co.) adjacent to the Mukwonago drainage or within the historic range of *Villosa iris* - the focus would be to look for additional extant populations of *V. iris*. *V. iris*, has only been recorded from the Mukwonago River (only reported location) , but may be present in other similar tributaries to the Ill. Fox River. Sugar Creek, Honey Creek , White River, and other tributaries to the Ill. Fox River should be surveyed to determine if *V. iris* is present and to examine habitat potential for expanding the range of *V. iris*.

2) Rock River (Rock, Jefferson and Dodge Co.) – Portions of the Rock River supported a historically diverse mussel community. A fish toxification program in 1976 called for the eradication of all fish from Hustisford dam to the lower dam in Watertown using antimycin. Little is known about present mussel community and the long term effects of these treatments. Impoundments, municipal runoff, and wastewater have likely had a significant impact on the mussel community. Potential for significant improvement. Large river system, will need non-wadable sampling protocol.

3) Little Wolf, Embarrass Rivers (Waupaca and Outagamie Co.) - Wolf River tributaries that we presently do not have a lot of information on mussels. Both rivers support a diverse mussel community, however, little is known about the species distribution within the systems. The purpose would be to determine range of *Epioblasma triquetra* in Wolf watershed - this could include Wolf mainstem, but we have a lot of records from there and if resources are limited I suggested focusing on the tribs. *E. triquetra* has been recorded from the Wolf, Embarrass, Little Wolf, and St. Croix Rivers. Preliminary sampling on the Little Wolf River in 2004 revealed a reproducing population at one location on the Little Wolf River, and a greater distribution than

previously reported. Distribution of *E. triquetra* within the Little Wolf River remains largely unknown. Limited sampling in 2003 and 2004 (Piette) indicated the Little Wolf River supports a highly diverse and dense mussel community.

4) Big Rib River and Eau Claire River (Marathon Co.) - Tributaries to the middle reach of the Wisconsin River, may be refugium for species no longer found in Wisconsin River due to past water quality issues. Big Rib River has suffered from past instream mining operations.

5) Sugar River (Dane, Green and Rock Co.) - This river was hit hard by illegal harvesting and would be good to see what is left. Non-point pollution may be impacting the mussel community. Good historical records for comparison.

6) Yellow River (Burnett and Washburn Co.) – Preliminary surveys in 2003 indicate high diversity, high density mussel beds. Species distributions within river are largely unknown. Extensive water pumping operations to draw down Shell Lake are likely to affect the mussel community and change substrate composition in the reaches below Spooner.

7) Baraboo River (Sauk Co.) - We have limited historic records and now that the dams are gone we might monitor it for recovery.

8) Sheboygan River and tribs (Sheboygan Co.) - but only those with suitable habitat remaining - we have a lot of historic records from this system and need to know what is there now, but a lot of habitat has also been damaged and may no longer be suitable.

9) Black River above Black River Falls (Jackson, Clark and Taylor Co.) - Limited data especially in the far upper reaches.

The following rivers have little or no information on their mussel communities.

10) Trempealeau River (Trempealeau Co.).

11) Manitowoc and Branch Rivers (Manitowoc Co.).

12) Grant River (Grant Co.).

13) Platte River (Grant Co.).

14) Big Eau Plain River (Marathon Co.).

15) Wisconsin River tribs below Prairie-du-Sac dam, habitat dependent.

Mussel Sampling Guidelines

Preface

This report is intended for use by biologists, resource managers, and field personnel when sampling mussels and mussel habitat in streams in Wisconsin. It contains protocols that should be used when sampling mussels and mussel habitat. Users should have a basic understanding and familiarity with mussel identification, mussel collection techniques, and stream habitat sampling techniques.

Throughout this document, we have presented what we have found to be effective approaches to estimating mussel communities and various stream habitat variables important to mussels. We give preferences based on our research and experiences. This document is by no means the final word on mussel and mussel habitat evaluation, and these guidelines may not provide the best approach in every situation. However, the goal of this document is to begin to standardize mussel and mussel habitat surveys conducted in Wisconsin. Standardized collection of mussel data will facilitate statewide comparisons of mussel communities and mussel habitat in streams, and will allow development of statewide mussel data.

Summary of Mussel Sampling and Reporting Protocols

The WDNR is currently reviewing freshwater mussel sampling methodologies for wadable streams in Wisconsin. The goal of this document is to clarify mussel sampling objectives and to provide a set of standardized mussel sampling and reporting protocols for wadable rivers and streams and wadable portions of large rivers. Protocols within this document were designed to collect mussel community and mussel habitat data over a broad range of stream habitats to be used to assess spatial and temporal changes of mussel communities. Mussel sampling protocols presented within this document were designed to provide meaningful mussel data given the constraints of limited resources of time and manpower. While these protocols represent a minimum amount of effort that should be conducted for mussels at sites, they may not be sufficient for some experiment objectives and more detailed or extensive surveys may need to be conducted.

The mussel sampling protocols established within this document are designed to answer three primary objectives associated with sampling mussel communities in wadable streams: 1) Are mussels present; 2) Which species are present; and 3) What is the density of mussels and habitat relationship. Three different sampling protocols were developed that can be used to answer the primary objectives. Protocol development was based on review of existing literature and our own research and experiences.

A historical record search should be conducted for a particular site or water basin to determine if mussels were present and to develop a species list prior to any fieldwork. If fieldwork is needed, mussel sampling should optimally be conducted during mid to late summer (mid June - late September) when stream levels are near base flows and water temperatures are near maximums. Initial searches are conducted to establish site locations and to determine if mussels are present. Initial sampling continues until mussels are found, or if no mussels are found, for one hour or until a maximum distance of 200 m is reached on streams < 7 meters mean stream width (MSW) and 300 m for streams > 7 meters MSW. If mussels are present at a site location, then more extensive qualitative sampling or quantitative sampling, or both is conducted. Qualitative (timed) search is conducted to establish species lists of mussels present at a site, but should not be used for mussel demographics. Qualitative searches continue for **two** hours (4 man-hours) or a maximum distance of 200 m for streams < 15 m MSW and for **four** hours (8 man-hours) or a distance of 300 m for streams ≥ 15 m MSW. Quantitative (quadrat) sampling is used to determine mussel demographics and habitat relationships. Quantitative sampling is conducted using a systematic random sampling design within defined grid areas, with sampling effort (area/m^2) increasing with stream size. Each grid area is sub-sampled using smaller 0.25 m^2 quadrats. Small rope grids 2.5 x 2.5 m (6.25 m^2) are used to sample streams < 7 m MSW, 14 grid areas are sampled with 8 sub-sample quadrats within each grid area. Large rope grids 5 x 5 m (25 m^2) are used on streams ≥ 7 m MSW, with 25 - 0.25 m^2 quadrats sub-sampled. The number of grid areas sampled increases with stream size. Streams between 7-14 m MSW will have 10 grid areas sampled, streams between 15-24 m MSW will have 15 grid areas sampled, and streams 25 m MSW and greater will have 20 grid areas sampled. Habitat measurements of variables important to mussels are taken at each grid area location. The number of live mussels, live less than 3 years old, and dead shells is recorded for each quadrat sampled within the grid area. Live mussels are measured for length, aged, sexed and examined for shell wear. Mussel data is recorded on standardized data forms to facilitate uniform reporting mussel information. Disturbance to mussels is sometimes be unavoidable, if impacts are unavoidable, mussels within

the impact area may be removed and relocated to safe habitat nearby. Collection and relocation protocols within this document are intended as a framework for the development of a Project Specific Relocation Plan.

Introduction to Sampling Methodologies

Freshwater mussels are an important component of freshwater ecosystems and are found in a wide variety of habitats within rivers, streams and lakes. Mussels communities are generally more diverse in flowing waters of creeks and rivers. Freshwater mussels represent one of the least understood, under appreciated, and under studied aquatic taxa. Freshwater mussels are long-lived relatively sedentary organisms susceptible to a wide variety of anthropogenic disturbances making them good biological indicators of stream condition and biological integrity. In particular, their often obligate link to other taxa (i.e. vertebrate, usually fish hosts) during their life cycle allow them to be sentry species for perturbations affecting a variety of species. In many cases, their full life cycles are still unknown. Habitat alterations caused by dams, watershed land use practices, construction activities and road crossings block host fish migrations, increase sedimentation and create changes in flow. In addition, water pollution and the introduction of exotic species also threaten freshwater mussels. All of these factors have led to declines in the native mussel fauna.

Indeed, freshwater mussels are the most imperiled of all faunas globally, 73 % of the mussel fauna are considered extinct or imperiled (Master 1992). In the United States and Canada, nearly half of the 297 mussel species are considered endangered, threatened, or extinct. In Wisconsin, 56% of the native freshwater mussel (28 of 50) species are listed as endangered (11), threatened (7), special concern (7), or extirpated (2) (WDNR 2004). Two species found in Wisconsin waters, Higgins Eye (*Lampsilis higginsi*) and Winged Mapleleaf (*Quadrula fragosa*) are listed as federal endangered, and four others are listed as federal special concern. It is imperative that resource managers take steps to determine status, distribution and abundance of freshwater mussels before more species become imperiled. An essential step in freshwater management and conservation is the development of standardized mussel sampling and reporting protocols.

The WDNR is currently reviewing freshwater mussel sampling methodologies for wadable streams in Wisconsin. The goal of this document is to provide a set of standardized mussel sampling and reporting protocols for wadable rivers and streams and wadable portions of large rivers. While this may seem like a straightforward task, it is quickly confounded by survey objectives, habitat types, and skill level of the collectors. Our goal is to clarify mussel sampling objectives and provide sampling methods that reduce sampling errors associated with habitat types and collector bias.

Many of the rivers and streams in Wisconsin have not been sampled for mussels. The most recent statewide mussel survey was conducted during the mid-1970's by Harold A. Mathiak (1979). Mathiak surveyed mussels at 641 sites on 251 rivers, providing a general distribution of mussels in Wisconsin. Certain caveats need be applied when interpreting mussel distribution based on Mathiaks survey. Sites were generally limited to where there was access, sampling effort varied from 15 seconds to 10 or more hours, and that most of his sampling occurred in shallow waters.

Until present, the methods used to collect mussels and mussel habitat information were left to the discretion of the individual biologists. As a result, methods were variable throughout the state; some biologist collected mussel data by using transects, some by random quadrats of various dimensions, and most often, simple random searches were conducted that varied in length, time and effort. Because of the differences in collection methods, there is little quantitative data available for use in comparisons of mussel populations across the state. Establishing a set of standardized mussel sampling protocols is a primary first step toward sound freshwater mussel management. In Wisconsin, standardized sampling protocols have been established for sampling fish, macroinvertebrates, and evaluating habitat for wadable streams, but do not currently exist for freshwater mussels. Standardized mussel sampling protocols will establish baseline data necessary for comparing mussel communities through time, and mussel distributions across the state. By using standardized sampling methods for quantitative and qualitative sampling, resource managers will gain a better understanding of mussel population trends and species distribution. One result of standardized mussel surveys may be a change in the status of some mussel species listed as endangered, threatened, or special concern. Some mussels may be found to be more abundant and widely distributed than previously estimated, while others may be less so.

Protocols within this document were designed to collect mussel community and mussel habitat data over a broad range of stream habitats to be used to assess spatial and temporal changes of mussel communities. Mussel sampling protocols presented within this document were designed to provide meaningful mussel data given the constraints of limited resources of time and manpower based on survey objectives. The following mussel sampling protocols are designed to collect a minimum level of mussel data in terms of area searched for quantitative searches or time spent searching for qualitative sampling based on the size of stream sampled. While these protocols represent a minimum amount of effort that should be conducted for mussels at sites, they may not be sufficient for some experiment objectives and more detailed or extensive surveys may need to be conducted.

Mussel Survey Objectives

Before beginning any mussel survey work, one must think clearly about the objectives of the survey. Is it to determine if mussels are present at a site? Is it to determine what species are present at a site? Is it to determine population densities and age structure? Or, is it to track changes in the mussel community over time? The survey objectives should be clearly outlined prior to initiating any mussel surveys. The mussel sampling protocols established here are designed to answer three primary objectives associated with sampling mussel communities in wadable streams. Each of the survey types outlined below are intended to meet specific objectives associated with mussel sampling (see Figure 1) and are intended to establish baseline information on mussel communities in wadable streams. A word of warning on mussel surveys, if population data surveys are needed, they must be conducted prior to disturbances associated with collecting a species list, therefore, for population data, emphasis should be placed first on quantitative sampling to evaluate mussel communities and then on qualitative sampling to establish a species list.

Objective 1: Are mussels present/absent.

Rapid qualitative (timed searches) sampling is used to determine if mussels are present at site location **YES** or **NO**? Establishes site location, general water quality, mean stream width. Rapid qualitative sampling techniques are used to determine if mussels (live or dead) are present. If mussels are absent, no additional surveys are necessary. If mussels are present, the next stage of sampling is initiated.

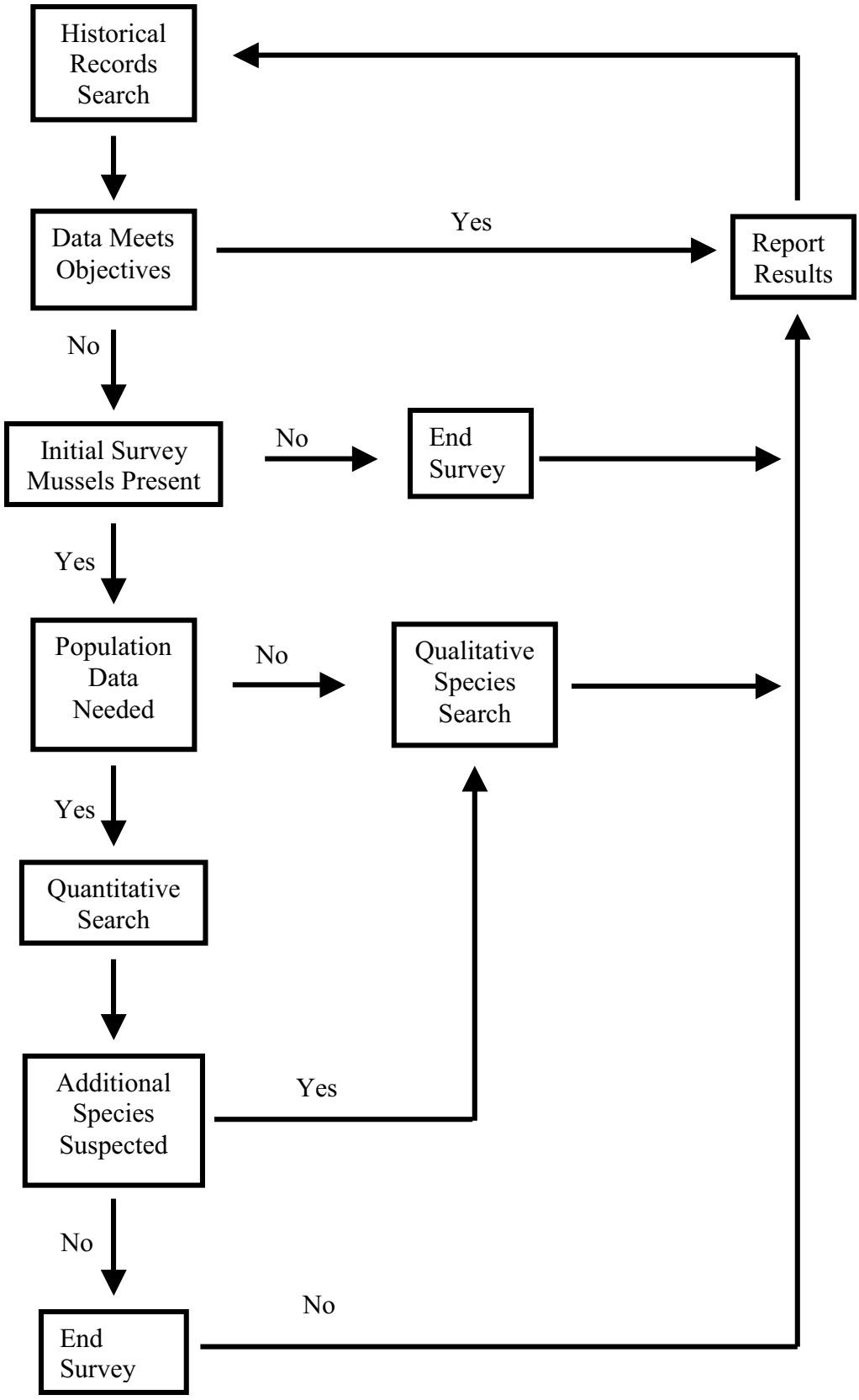
Objective 2: Which species are present.

Qualitative (timed search) sampling establishes a species list of mussels found for a given amount of effort (man-hours). Sampling methods are designed to cover large areas of the streambed and to search multiple habitat types, targets mussel species that may be present in very low densities such as species listed as special concern, threatened, or endangered. Establishes mean stream width, and general habitat data for stream area covered. Timed searches should not be used for population demographics (e.g., density, relative abundance, age structure, or temporal change).

Objective 3: Comparing mussel populations, and assessing temporal change.

Quantitative (quadrats) sampling is used to assess temporal change in mussel populations and should be used with before-and-after surveys to determine effects of perturbations or changes in stream habitat on mussel populations. Quantitative sampling methods are used to determine mussel densities, age structure, and habitat relations of the mussel community. Methods used will allow comparison of mussel communities between sites, across basins, and statewide. Reduce collector bias and sampling error by using 0.25 m^2 quadrats within a defined grid area. Collect mussel habitat data and determine mussel habitat relations. Provide valid statistical framework for repeatable collection techniques after a time period. Establish mussel densities and species list for a given amount of sampling effort in terms of m^2 searched. Quantitative sampling may miss low density mussel species or species associated with unique habitats, and may be used in conjunction with qualitative (timed search) sampling to establish a comprehensive species list for sites.

Figure 1. Flow chart for mussel surveys.



Historical Search

Prior to any fieldwork, a records search should be conducted to determine if historical mussel information already exists for a particular site or water basin. This information can be used to develop a list of mussel species historically known from the water basin. Note any endangered, threatened or special concern species and their general habitat preferences (WDNR 1999). Historical data should only be used as an indicator of mussels that have been found in past surveys and not as an indicator of species absence. The following sources can provide information on mussel species that may be present within state waters, but should not be viewed as comprehensive or all-inclusive for any given body of water, please note, mussel data has often not been published.

Mathiak, Harold A. 1979. A River Survey of the Unionid Mussels of Wisconsin 1973-1977. Published by Sand Shell Press, P. O. Box 44 Horicon, WI 53032.

Wisconsin Department of Natural Resources - Mussel Atlas. The WDNR Mussel Atlas is currently under development as the main database for storing mussel data from historical and current mussel surveys.

Wisconsin Department of Natural Resources – Bureau of Endangered Resources Natural Heritage Inventory database. This database is used to track rare species found in Wisconsin and is available through the Portal, http://intranet.dnr.state.wi.us/int/land/er/nhi_portal/index.htm

Wisconsin Department of Natural Resources. 2004. Wisconsin Natural Heritage Working List. Wisconsin Natural Heritage Program, Bureau of Endangered Resources. This list is updated annually, internet link http://dnr.wi.gov/org/land/er/working_list/taxalists/mussels.htm

Museum records or other literature including survey reports in the gray literature.

General Sampling Procedures

A malacologist experienced in unionid collection will coordinate all sampling efforts. The malacologist must be familiar with the identification of unionid mussel fauna and have particular expertise in the identification of rare species. Mussel sampling is strongly influenced by collector experience, we recommend that experienced field crews be used to collect mussel data. Experienced collectors are often able to collect a greater number of individuals and species, especially small and cryptic colored specimens, when compared to untrained collectors.

Our mussel sampling guidelines were designed for permanent wadable (most areas < 1.2 m deep) streams. Mussel sampling in wadable streams should optimally be conducted during mid to late summer (mid June - late September) when stream levels are near base flows and water temperatures are near maximums. Sampling during this time period when mussels are active will allow mussels disturbed during sampling to re-establish themselves in the substrate. Sampling outside of this optimum period may be necessary, but water temperature should be at least 40^{o}F, to minimize thermal stress to mussels. In addition, summer sampling during peak water temps is more comfortable for collectors snorkeling or diving, allowing for longer sampling periods.

Station locations

If no recent or historical mussel information exists for a given stream, a preliminary float or walk may be necessary to establish station locations and collect general habitat information. Station locations should be representative of available habitat within the sampling reach and should be located some distance away from permanent structures that may influence mussel distribution (e.g. bridge abutments, dams), unless the objective is to evaluate these sites or the effects of these structures. Caution is advised when establishing sampling stations to avoid investigator bias toward habitat types, mussels are often found in unexpected habitat when viewed from above. If a stream has well-developed pool riffle structure, each station should start at the base of a riffle. Mussel species richness and density are often higher at the head and base of riffle areas, and in moderate run habitat with stable mixed substrates. If the objective is to compare between reference and impacted sites, then similar habitat types should be sampled between sites. Enough stations should be established to give adequate longitudinal coverage of the selected stream reach. Stations should be located above and below major barriers to fish migration if whole stream reaches are being evaluated. A good sampling design to establish station locations will eliminate some of the common pitfalls (sites representative of available stream habitat; spatial resolution of mussel distribution) associated with mussel sampling (Strayer and Smith 2003). Initial surveys should be used to establish station locations only, and should not be used for species richness or density. Once station locations have been established, qualitative sampling to determine which species are present, or quantitative sampling to obtain population demographics should be conducted depending on survey objectives.

Station Location Data Sheet (* indicates required fields**)**

A station data sheet is required for each mussel sampling location regardless if mussels are present. This sheet summarizes location, water characteristics, and general habitat characteristics for the station. A station summary data sheet is to be filled out each time a site is sampled. The parameters on the station location data sheet are as follows:

Location **----------**

Waterbody ID: A unique seven-digit number identifies each stream (all streams, rivers, and lakes in Wisconsin). All waterbodies have or should have an assigned number. These numbers are available on the WDNR Intranet, under the listing for "DNR Tabular Database Service" for the WDNR Register of Waterbodies (ROW). As with Stream Name, Waterbody ID should be the same for all stations on a stream.

*Stream Name: The name of the stream as shown on the most recent USGS 7.5' topographic map. USGS maps can be accessed on the WDNR Intranet. The stream name used here should be identical to that used on all other data sheets, and to that used for all other stations on the same stream. Make sure the spelling of the name is accurate and includes all parts of the stream name (e.g., "West Branch", "Middle Fork", "River", "Creek", "Brook", "Run", etc.) to avoid confusion. Other commonly used names for the stream can be written here in parentheses.

Station Name or Number: Name of station on stream, if numerical, station 1 should be located at downstream most station, the next upstream is number 2, and so on.

Site Mile: The reporting of this parameter is optional. The distance along the stream channel from the mouth of the stream to the downstream end of the station. This distance is a useful shorthand for indicating and identifying the location of the station. Site mile should be measured on the most recent USGS 7.5' topographic map to the nearest 0.1 mile using a map measurer.

*Date: (mm/dd/yyyy) Fill in the date when the mussel data were collected for the station.

*Collectors: Person or persons collecting mussel data, list primary investigator first.

*County: The name of the county in which the station is located.

*Township, Range, Section, ¼ - ¼ Section, 1/4 Section: Legal description for the starting location of the station within the Public Lands System. These can be determined from recent USGS 7.5' topographic maps or a detailed county map. On a topographic map, a "land locator" template is useful for determining the ¼ - ¼ and 1/4 Sections, which are indicated by a compass direction (NW, NE, SW, or SE). Note that in Wisconsin, all Townships are "N" (north), but Range can be either "E" or "W" (east or west). Make sure the appropriate letter is included for both Township and Range. Please note, either TRSQ or GPS must be included, both is best.

*Station Start GPS Location: GPS Latitude and Longitude of station downstream end starting location. If using hand held GPS units use decimal degrees (hddd.ddddd) (e.g. N 045.79330; W091.96815). It is important that geographic coordinates of the **start** of the station are recorded, along with the method used to determine latitude and longitude (e.g. USGS map, mapping software, global positioning system (GPS) units). The geodetic Datum Used upon which the coordinates of the map or GPS coordinates are based (e.g. North American Datum 1983 (NAD 83)) should also be recorded. Datum for USGS topo maps are shown on the map legend.

Nearest Road/Access: Name of nearest road or access point to station starting location.

Dist. from Access: Distance from access point to starting location measured along stream centerline, record units in (m) if < 1 km and (0.1km) if > 1 km.

*Map (to accompany data): Copy of topographic or comparable map with sample site noted. If an entire stream reach is sampled, a larger scale map may be used, with all sample sites noted.

Water characteristics----------

All water characteristics should be measured in water of moderate current at least 0.15 m above the bottom and 0.15 m below the surface (if possible).

*Time: The time (in "military" format; i.e., 9:30 AM is 0930 hours and 9:30 PM is 2130 hours) at which measurements of water characteristics are made.

*Water Level: An estimate of the level of the stream at the station. Check the appropriate category, and measure the vertical distance (nearest 0.1 m) if "Above" or "Below" normal. If there are areas of stream bed that are dry but look as if they would normally be underwater, then the water level is "Below"; measure the vertical distance between the current water level and the "Normal" water level. If the stream is flowing over or through areas that have terrestrial vegetation (e.g., grasses, forbs, willows, but not bulrushes and cattails) then the water level is

"Above"; measure the vertical depth of water above the normal water line. Otherwise, the water level is "Normal" (at or near baseflow). **Sampling should not occur if the water level appears to be substantially above normal, unless necessary.** Mussels are difficult to see at normal water levels; increased depths and reduced visibility from turbidity greatly reduce the chance of finding mussels.

Note: Channel characteristics rather than the amount of precipitation in the recent past should be used to determine water level. Streams with a high proportion of ground water input may retain normal flows well into drought periods. Conversely, such streams may show little response to heavy rains, particularly if the local water table has been greatly lowered by prolonged drought. On the other hand, streams that are runoff dominated may fluctuate greatly in water level in response to short-term wet and dry periods.

*Air Temp: If possible, measure air temperature during the warmest part of the day to estimate maximum values. Take the air temperature in the shade with a dry thermometer; evaporation from a wet thermometer will lead to a measured air temperature lower than the true value. Measure to the nearest 1 degree Celsius.

*Water Temp: Take the water temperature in mid-channel, during the warmest part of the day to estimate maximum values, if possible. Measure water temperature away from any large objects that project above the surface. Such objects may act to efficiently transmit heat and influence local water temperature. Avoid areas of the stream where subsurface or bank springs may be present. Measure to the nearest 1 degree Celsius.

Conductivity: Measure with a high-quality electronic meter. Most conductivity meters have built-in automatic temperature compensation to 25 °C (77 °F), but this should be confirmed before using the meter. On some older meters the temperature compensation must be set by hand, and on others, there is no compensation. For the latter meters, conductivity at 25 °C can be calculated using procedures outlined in "Standard Methods for the Analysis of Water and Wastewater", a book available at many WDNR offices. Whatever meter is used, it should be calibrated before every use. Measure conductivity in umhos/cm.

Turbidity: The reporting of this parameter is optional. If reported, measure with a high-quality electronic meter, which should be calibrated before every use. Measure and report turbidity in nephelometric turbidity units (NTUs).

*Water Clarity: Record whether the water is **Clear**, **Stained** due to dissolved organic compounds, **Silty** due to relatively light loads of fine sediment, or **Turbid** from heavy loads suspended sediment.

*Visibility: Record depth to nearest (cm) to which small substrate (fine gravel) can be clearly seen when viewed from directly above. Mussel detection during visual searches and search times are strongly influenced by water clarity.

*Gradient (flow): A general description of overall water flow at station location, different from actual flow measurements. Circle "**N**one" if no visible flow is present. Circle "**L**ow" if visible flow present, pools and slow moving runs common. Circle "**M**oderate" if water is swiftly

moving, with runs and slow riffles present, but has few riffle/rapids areas. Circle "**H**igh" if riffle/rapids areas common, broken water surface abundant.

*Natural or Tailwater: Check tailwater if site is within 5 miles below a dam. Record distance below dam (0.1 miles) to station start, use USGS maps for distance.

Sampling Strategy ----------

*Sampling Strategy: Record type of sampling method used to survey site on that date (e.g. initial, timed search, quadrats (qualitative).

*Search Times: Use military format (2400). Record actual search times, start time is when searchers first start looking for mussels. Record end time when searchers stop looking for mussels. Do not include time spent on gear preparation.

*Area Searched: Record total length (m) of upstream distance searched. Measure distance along stream centerline. Record mean width of stream. Use stream width measurements to obtain mean stream width (round down to the nearest meter). This value is used to determine the length of stream to sample, number, and size of grid areas for quantitative sampling.

*Bank: Record if only **L**eft or **R**ight bank or **B** for both, if stream was searched from bank to bank

*Mussels Present: Were mussels found at station location **Y** or **N**.

*Distance to live mussels: Record the upstream distance (m) to first live mussel found. Measure distance along stream centerline.

General Habitat Description----------

*Stream Widths: These 10 spaces are provided for the determination of Mean Stream Width. Ten preliminary measurements of stream width (round down to the nearest meter) throughout the approximate station length should be made to determine the MSW (use a measuring tape on small streams, laser range finder on large streams). These measurements should be taken at different points to incorporate the variation of pools, riffles, and runs. Stream widths may be taken while conducting the initial search to avoid disturbing visibility.

*General Habitat Description: General habitat descriptions are subjective measurements and should not be considered quantitative. General habitat descriptions will allow collectors to evaluate site conditions and help reduce the need to conduct field reconnaissance in future mussel surveys. General habitat descriptions are not intended for true habitat evaluations, if habitat evaluation is the objective, follow methods in WDNR publication **"Guidelines for Evaluating Fish Habitat in Wisconsin Streams"** (Simonson et al. 1993). General habitat information is recorded after conducting the initial search

*Macrohabitat: The amount of general habitat is measured along the stream centerline (m) for each different type of habitat. Habitat types should be recorded as follows:

Pools: Areas of the stream with deeper than average maximum depths, with no obvious surface turbulence or broken water. Water velocities are always slow. The longitudinal profile of the streambed in a pool is often bowl shaped. "Pocket water" refers to groups of small pools located behind boulders or other obstructions to flow, often in areas of otherwise fast or turbulent flow.

Runs: Areas of the stream with average maximum depths and little or no surface turbulence. Water velocities may be fast or slow, but the water surface appears generally smooth. Runs with slow velocities are sometimes called glides. During droughts, many shallow runs may become riffles.

Riffles: Areas of the stream characterized by shallower than average maximum depths and obvious surface turbulence. Water velocity is faster than average. During high flows some riffles may become runs.

Rapids: Areas of the stream characterized by shallower than average maximum depths and obvious surface turbulence. Water velocity is faster than average, broken water surface, whitewater.

Other: Areas of the stream that do not fall into the above general categories. List other habitat type.

*Substrate: Record the amount of substrate type present at each station. Substrate is recorded as a percentage (nearest 5%). Substrate types should be recorded as follows:

Detritus: Partially decayed organic matter such as leaves, sticks, dead macrophytes, etc. When very fine, may appear similar to silt.

Clay: Very fine inorganic material; individual particles barely or not visible to the naked eye. Either dark brown or gray in color. Feels gummy and sticky in hands; slippery when underfoot. Retains shape when compacted, and partially or completely supports a person's weight when it makes up the stream bottom. Maximum diameter of 0.00024 - 0.0005 mm.

Silt: Fine inorganic material, typically dark brown in color. Feels greasy and muddy in hands. Loose; does not retain shape when compacted into a ball. Will not support a person's weight when it makes up the stream bottom. Maximum diameter of 0.004 - 0.061 mm.

Sand: Inorganic material smaller than fine gravel but coarser than silt. The material found on a beach. Maximum length of 0.062 mm - 1.9 mm.

Gravel: Rocks with a maximum length of 2 mm - 64 mm.

Cobble: Rocks with a maximum length of 65 mm - 260 mm.

Boulder: Rocks with a maximum length of 261 mm - 4.1 m.

Bedrock: Solid, uniform rock bottom.

*Instream vegetation: Record as a percentage (nearest 5 %) the amount of different types of vegetation present in the stream Channel where:

Emergent: A visual estimate of emergent plants (rushes, cattail, arrowhead) within stream channel, do not count inundated grasses.

Submergent: A visual estimate of submerged aquatic plants present within the stream channel.

Algae: A visual estimate of attached and filamentous algae within stream. Filamentous algae is algae attached to the bottom or banks that forms long filaments, and Attached Algae is algae attached to the bottom or banks that forms a mat or crust, but does not form long filaments. Abundant growth identifies streams polluted with excessive nutrients (usually nitrogen and phosphorus).

*Riparian land use: The amount of various land uses on both banks. In baseline habitat evaluations "banks" are defined as the land from the edge of the stream at normal water level to a point **5 m inland**, following the contours of the land. This definition avoids confusion in identifying the actual banks. Visually estimate each category listed below (nearest 5%) for both banks combined. **The sum of estimates must equal 100 %.** If a category listed on the sheet is not present, enter a zero for that category. If a category that is not listed on the sheet is present, specify the identity of that category and list the percentage next to "Other". The listed categories are as follows:

Wetland: Low-lying land that is covered with standing water for much of the year.

Meadow: Land dominated by grasses and forbs with few woody plants, which is not subject to regular mowing or grazing by livestock.

Woodland: Land dominated by trees (either coniferous or deciduous), most of which are taller than 3 m.

Pasture: Land that is regularly grazed by livestock.

Cropland: Land that is plowed and planted with crops on a yearly basis or is regularly mowed for hay.

Developed: (Commercial/Residential/Urban): Includes all buildings and lands that have been modified for human use, and all roads (paved and unimproved), railroads, paths > 2 m wide, parking lots, yards, etc. Also, parks, playgrounds, golf courses, ball fields, parking lots, etc.

*Artificial bank structures: Are recorded in actual length (m) of stream bank covered for both sides of stream, note length along with stream side, when present.

RipRap: Quarried stone or fieldstone placed along bank to prevent erosion.

Seawall: Solid wall or barrier (concrete, stone, wood, or other material) with vertical or very steep slope placed along bank to prevent erosion.

Other: Manmade or deposited materials placed along bank to prevent erosion. Describe other type of artificial bank structure.

Initial Survey

A preliminary mussel search is conducted to determine the presence or absence of mussels at a site. If prior surveys have determined that mussels are present, then an initial survey need not be conducted. Instead, a quantitative survey to collect mussel population data or a qualitative survey to determine which species are present should be conducted. Initial searches are used to determine if freshwater mussels of any species are present at a location, the objective of initial searches is simply to determine if freshwater mussels are present. Initial searches may end prior to the time limit once mussels (live or dead) are found at a location to save time and allow more sites to be searched, or more extensive sampling at a site. Initial searches are not intended to be used to establish species lists. Data collected during initial searches should be recorded on the **Station** data sheet. Initial searches are conducted using visual searches of shallow water and near shore areas in streams to locate live mussels or dead shells.

Initial searches are relatively rapid searches. Ideally, two persons each equipped with a mask-and-snorkel should conduct the initial search. Each person should select a shoreline and begin searching in an upstream manner quartering back and forth towards the center of the stream beginning at the station starting point. Initial searches are qualitative methods designed to cover large areas of stream. Searchers may use waders to conduct initial searches in shallow streams, but the use of waders may restrict the depth of sampling especially when doing tactile (hand grubbing) searches. Searchers need not use mask-and-snorkel in very shallow areas where the substrate is clearly visible (e.g. sand flats), to facilitate greater coverage of the site area. A mask-and-snorkel is recommended for deeper water areas were the bottom substrate is not clearly visible. In turbid water where the bottom is not visible, hand grabbing (short tactile searches sweeping the hands back and forth while sifting through the substrate to feel mussels) should be conducted randomly while progressing upstream. Use of underwater writing slates to record data or having a third person recording while others search is also recommended. On small streams less than 4 meters wide, one person may cover the entire stream.

Initial searches should continue until mussels are found, or if no mussels are found, for one hour or until a maximum distance of 200 m is reached on streams less than 7 meters mean stream width (MSW) and 300 m for streams greater than 7 meters MSW. If tactile searches are necessary due to high turbidity, search time should be limited to one hour. If the search time limit is reached prior to the distance limit, record the total mid-channel distance searched. Record the number of searchers, time, and distance sampled. Record the distance searched to where first live mussels were found. This distance can then be used to establish the starting point for quantitative or qualitative mussel sampling. If no mussels are found, then species found is recorded as **No Mussels**. If mussels are present, record all species found using the scientific name (e.g. *Anodonta grandis*), including dead shells. Use scientific name to avoid confusion of mussel species, many species have been given several common names which may vary between collectors and locality. As an example, *Strophitus undulatus* is commonly called creeper,

bankclimber, or squawfoot, which could be confused with *Lasmigona costata* sometimes called squawfoot or *Lampsilis teres* occasionally called creeper. All live mussels should be handled gently and once identified re-bedded immediately back to the location where they were found.

Qualitative Survey

Qualitative (timed) searches are conducted to establish species lists of mussels present at a site. Qualitative searches should not be used for quantitative purposes (e.g. population density, abundance, age structure, habitat association). The probability of detecting a mussel species during a timed search varies greatly depending upon mussel species, field conditions, collector experience, and length of time spent searching (Strayer et al. 1997). Typically the largest and most visible mussels are collected while small species, juvenile, buried, and cryptic colored species are often overlooked. Qualitative searches are more intensive than initial searches. Qualitative sampling is used to establish a species list and to determine if species are present for a given amount of sampling effort (person-hours or distance). Timed searches are sometimes more efficient at finding rare and low-density species compared to quantitative sampling and may be used to augment quantitative sampling to compile a more comprehensive species list for a site. However, because of the informal sampling design associated with timed searches, there is no valid way to assess sampling variation, thereby limiting their usefulness to assess mussel populations (Vaughn 1997; Strayer and Smith 2003).

Mussel species richness is a function of stream size and generally increases with increased drainage area (Strayer 1983; Watters 1993; Piette, unpublished data). The amount of effort to develop a species list for a site also increases with increasing stream size. Preliminary sampling during protocol development determined that mussel species richness increased rapidly during the first hour of searching and approached an asymptote after two hours of sampling on smaller streams and four hours on larger streams. The increase in sampling effort with stream size corresponds to the increase in substrate area searched and the likelihood of more diverse mussel communities. In addition, a greater amount of time is generally required to detect the presence of rare species. As a minimum, qualitative searches should continue for **two** hours (4 man-hours) or a maximum distance of 200 m for streams less than 15 meters MSW and for **four** hours (8 man-hours) or a distance of 300 m for streams greater than 15 meters MSW.

Preferably two persons should conduct the qualitative search. Use of mask-and-snorkel is recommended. Each person should select a shoreline and begin searching upstream quartering back and forth towards the center of the stream beginning at the station starting point. Search patterns during qualitative searches are slower and more deliberate than initial searches to look for rare, small, or cryptic species. Searchers should stop occasionally in areas of loose or fine substrate and hand grab (short tactile searches sweeping the hands back and forth while sifting through the substrate) to detect mussels not visible at the substrate surface. Areas of mixed loose gravel should be fanned occasionally to detect mussels hidden between the substrate. Searchers need to explore all types of habitat including banks and backwater areas to locate species that prefer these habitats (e.g. *A. ferussacianus*, *P. grandis*, and *S. undulatus*). Dense mussel beds maybe found in areas with moderate vegetation. These areas need to be carefully searched by hand grabbing to locate mussels hidden within the vegetation. Streams with low visibility due to turbidity will require hand grabbing to locate mussels. Streambanks may also be examined to look for dead shells or midden piles, species not found alive, may be represented by dead shells.

Record number of searchers, sampling time (min) and distance covered (m). Record all species found (live and dead) using scientific name, number of live individuals (noting number of live individuals 3 years old or less separately) for each mussel species found on the **Timed Search** datasheet. The number of live individuals less than three years old is used to indicate if there has been recent recruitment of a mussel species. Do not count the number of dead shells of individual species but do record any dead species not found alive.

Searchers may want to use underwater writing slates to record data or have a third person recording. Slates can be made from opaque white plexiglass (Figure 2). All live mussels should be handled gently and re-bedded immediately back to the location where they were found once identified. Replacing mussels back into the substrate immediately where they were found reduces handling time at the end of collection, minimizes the disturbance to mussels, and reduces the risk of mussels being replaced in the wrong habitat. Mussels that are not readily identified can be placed in mesh bags for later positive identification. All mussels bagged for later identification should be replaced back to their appropriate habitats. Listed species (special concern, threatened, endangered) should be measured for length, sexed if possible and aged to provide information on population viability.

Qualitative Sampling Procedure

If mussel population data is not needed or requested for a site, qualitative (timed search) sampling can be used to establish a species list for a site. If population data is needed or requested, then qualitative sampling should be conducted after quantitative (quadrat) sampling is completed, especially if additional species have been reported, or are suspected for a site but were not found during quantitative sampling. Qualitative sampling should be conducted using two searches to locate mussels and a third person to help identify mussels and record mussel and habitat data. The following procedures should be followed for qualitative sampling:

Establish station start location. Stating location should be representative of available habitat within the sampling reach and should begin at the base of a riffle or in run habitat.

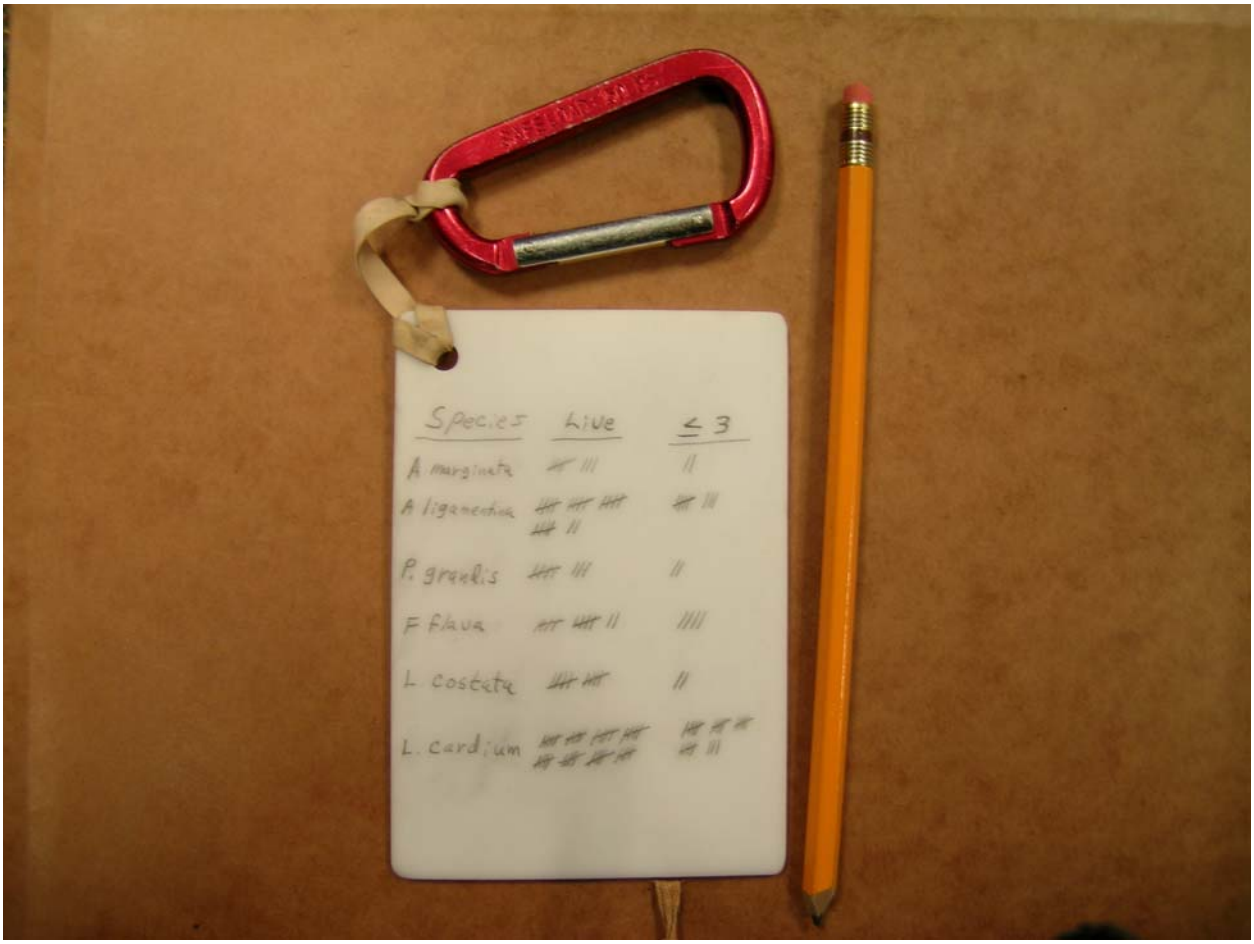
Complete mussel **Station** data sheet each time a site is sampled.

Use MSW established during the initial survey to determine length of stream to sample. If MSW has not been established, measure and record stream width at starting location and then approximately every 20 meters apart on streams less than 15 meters wide and 30 meters apart on streams greater than 15 meters wide. Use a tape measure for small streams and laser range finder (if possible) for larger streams.

Record start time on **Timed Search** data sheet and begin looking for mussels. Search times are 2 hours for 2 searches (4 man/hrs) for streams less than 15 meters MSW and 4 hours for 2 searchers (8 man/hrs) on streams 15 meters and greater MSW.

Record all species found using scientific name (e.g. P. grandis), including those represented by dead shells (do not count dead shells). Count and record all live mussels found by species. Count and record all mussels found less than 3 years old by species. Data can be

Figure 2. Underwater writing slate made from opaque plexiglas used during mussel collecting. Column headings include species name, number live, and number of live individuals equal or less than 3 years old. Length, sex and age of rare species are recorded on back of slate. A metric ruler is also scribed onto the backside of slate for measurements.



recorded on small underwater writing slates attached to wrist. All live mussels collected listed as endangered, threatened, or special concern are identified, measured for length, aged, sexed and coded for shell wear on **Mussel Length Age** datasheet. Mussels not identified should be clearly photographed or a voucher specimen brought back to lab for positive identification by a malacologist familiar with regional fauna. Uncertain identities should be photographed and experts consulted or dead shell retained.

Record stop time when time limit or when distance limit has been reached (2 hr or 200m for streams < 15 m MSW and 4 hr or 300 m for streams ≥ 15 m MSW).

Sum mussel data for species found, number live, number live 3 years old or less, and species represented by dead shells only.

Record general habitat data on **Station** datasheet. Measure and record length (m) of macrohabitat for search area. Estimate and record substrate percentages, macrophyte and algae coverage, and riparian land use. Measure and record length (m) of artificial bank features for each bank.

End survey.

Quantitative Survey

Quantitative (quadrat) sampling methods are used to determine mussel populations, densities, age structure, and habitat relations of the mussel community. Methods used were designed to provide a valid statistical framework and will allow comparison of mussel communities between sites, across basins, and statewide. Quantitative sampling can be used in before-and-after surveys to determine effects of perturbations or changes in stream habitat on mussel populations. Quantitative sampling is recommended for obtaining density, abundance, age structure, and mussel habitat associations. Sampling using 0.25 m^2 quadrats is more effective at finding buried mussels, juveniles, small, and cryptic colored species often missed during other searches (Vaughn et al. 1997). However, quantitative sampling may not be effective at detecting rare low-density mussel species. Quantitative mussel sampling should be conducted once initial sampling has been done and sampling stations established, **prior** to doing any qualitative sampling that may disturb mussels.

On wadable streams, quantitative sampling is conducted using a systematic random sampling design within defined grid areas, with sampling effort (area/m^2) increasing with stream size. This sample design was selected for repeatability and ease of implementation in the field. Systematic sampling within defined grid areas during protocol development provided precise estimates and good spatial coverage of sites. Use of grid areas simplifies locating sampling units, and provides better repeatability for long-term monitoring of mussel population. It is much easier to locate a 5 m grid area to subsample than it is to locate individual 0.25 m^2 quadrats. Grid areas are relatively quick to setup and take habitat measurements from (5 – 7 min), and provide a fixed area from which mussel densities can be estimated and related to habitat. Use of mask-and-snorkel is recommended, and SCUBA gear may be necessary for mid-channel sampling at deeper (>0.75 m) sites. SCUBA gear allows the collector to remain near the substrate for easier observation of mussels within quadrats.

Sampling Grids

Sampling grids are constructed from rope with knotted loops on each corner for anchor stakes and a center rope in the middle (Figure 3). Grids may be made from ¼ inch brightly colored braided polypropylene rope for increased visibility and ease of handling. The perimeter and center rope should be marked at 1 m intervals (suggest brightly colored tuffs of rope or twine) to facilitate quadrat placement within the grid. Make sure ropes are marked equally. Small grids measure 2.5 m between corner knots and large grids measure 5 m between corner knots. Sampling grids are further divided into 25 sampling units for small grids and 100 units for large grids. Grid areas are subsampled using 0.25 m^2 quadrats with placement of quadrats within the grid area being randomly selected. For 2.5 meter grids, eight random location are selected from 1-25 with no duplicates. For 5 meter grids, 25 random locations are selected from 1-100 with no duplicates. Sampling units are numbered from left to right and bottom to top. Search times to collect mussels within grid areas is variable, and generally increases with mussel density, turbidity, vegetation, and substrate complexity.

Sampling Quadrats

Grid areas are subsampled using a 0.25 m^2 (50 cm x 50 cm inside measurements) quadrat (e.g. metal bar stock or weighted PVC pipe). Quadrats should weigh enough to remain in place on the substrate in stronger currents. We recommend using 1" x ¼" metal bar stock to construct quadrats for durability and weight. Quadrats should be brightly painted (yellow works well) to increase visibility underwater. Once in place, quadrats are visually searched for mussels. In turbid waters, use a light tactile sweep across the quadrat to locate mussels. Quadrats are then lightly fanned to remove fine substrates and reveal buried mussels. All quadrats should be fanned to a depth of 5 cm to expose buried mussels. In heavy macrophytes, the plant stems and leaves can be cut away after the first visual or tactile search is finished. All mussels found inside quadrat area should be placed in a mesh bag numbered with the corresponding quadrat location. Numbered bags can then be handed to non-searching personnel for identification and measurements.

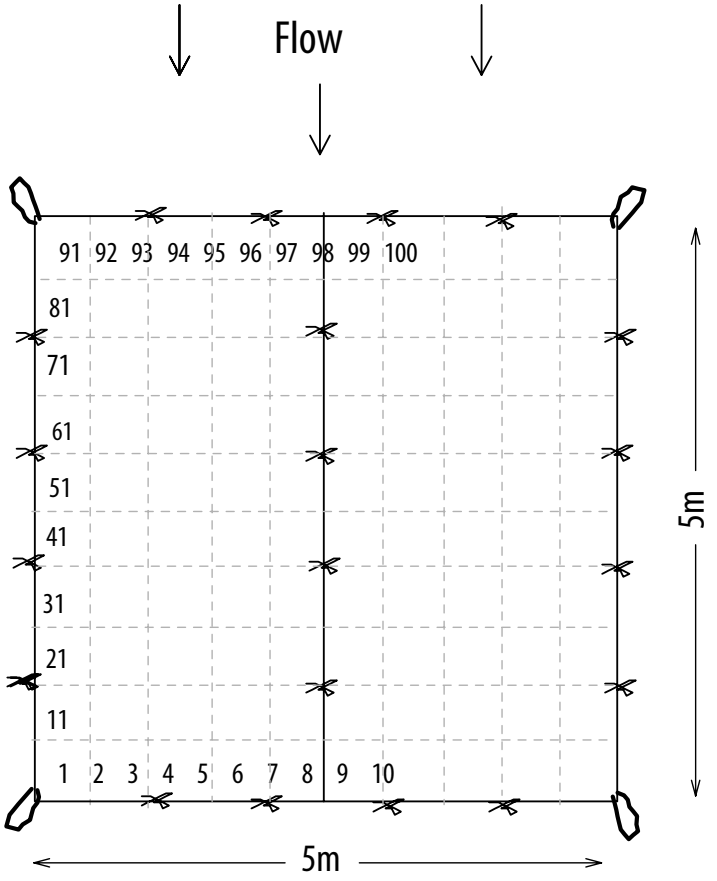
Sampling Grid Placement

Placement of grid areas depends on stream size (Table 1) and will be randomly selected for starting point and starting position within the stream channel. Data sheets that establish ramdom starting point and grid locations can be compiled quickly using Excel© or similar software.

Table 1. Stream size category, number of grid areas, grid sequence, number of quadrats, and area sampled for mussel sampling in wadable streams.

Stream size	Grid size	N. of grids	Grid sequence	Quads/grid	Total quads	Area sampled
<7 m	2.5 m	14	L-R	8	112	28 m^2
7-14 m	5.0 m	10	L-R	25	250	62.5 m^2
15-24 m	5.0 m	15	L-R-M	25	375	93.75 m^2
>25 m	5.0 m	20	L-M^1-R-M^2	25	500	125 m^2

Figure 3. Diagram of 5 x 5 m rope grid area with 0.25 m^2 quadrat numbering locations and imaginary grid lines for quadrat placement used in quantitative mussel sampling. Quadrat location 1 is always located in the downstream left-hand corner of the grid area.



On small streams less than 7 m MSW the start of sampling locations will be randomly selected from 1 to 5 m above the station start. Small rope grids 2.5 x 2.5 m (6.25 m^2) are used to sample streams less than 7 meters MSW, 14 grid areas will be sampled with 8 quadrats sampled within each grid area. Preliminary sampling during development of this protocol revealed 8 subsamples in a 2.5 x 2.5 m area for small streams gave a reasonable representation of the mussel community. Grid areas are sampled in sequenced pairs of **L**eft and **R**ight bank locations, seperated by 2.5 m (upstream distance). The first bank location for a pair of grids is randomly selected for L or R bank, and then the opposite bank is sampled before selecting another random bank location of L or R. Datasheets that establish random starting distance, bank location (Table 2) and quadrat locations (Table 3) should be made up prior to fieldwork.

On larger streams 7 m MSW or greater, the start of sampling locations will be randomly selected from 1 to 10 m above the station start. Large rope grids 5 x 5 m (25 m^2) are used to sample streams 7 m MSW or greater. Grid areas are separated by 5 m (upstream distance). A total of 25 quadrats will be randomly sampled within each grid area. The number of grid areas to be sampled for each stream site increases with stream size. Streams between 7-14 m MSW will have 10 grid areas sampled, streams between 15-24 m MSW will have 15 grid areas sampled, and streams 25 m MSW and greater will have 20 grid areas sampled. The number of grid areas increases with stream size to provide good spatial coverage of a stream site and to incorporate different habitat types. Preliminary sampling during development of this protocol indicated 25 subsamples within a 5 x 5 m area for large streams gave a reasonable representation of the mussel community. Habitat information is collected within each grid area to establish mussel habitat associations.

On large streams, grid placement within the stream runs in sequenced groups of two, three, or four grid areas, dependent upon stream size. The first grid in a group is randomly selected for channel location and then the remaining grids in the group are sampled in order before selecting another random channel location. Datasheets that establish random starting distance, bank locations (Table 4, Table 5, and Table 6) and quadrat locations (Table 7) should be made up prior to fieldwork. Grid area data sheets can be compiled quickly using Excel© or similar software. Grid placement on streams less than 15 m MSW will be randomly selected for L or R bank (Table 4). Grid placement sequence for streams between 15-24 m MSW will be L, R then M (Table 5) and for streams 25 m MSW or greater, placement will be L, M^1, R then M^2 (Table 6).

For example, in a 17 m MSW stream, if the random start is 4, sampling would begin a distance of 4 m above the station starting point. If the first random bank location selected is **L** the grid would be placed long the left bank, the next grid would be placed **R** (right bank) 5 m above the first grid area, and the last grid in the rotation would be placed **M** (mid-channel) 5 m above the right-channel grid (Figure 4). Record distance to left bank for left bank and middle grids and to the right bank for right bank grids. Once all three locations have been sampled a new random bank location is selected. If right were selected, the order would be **R**, **M**, and then **L** and so on, until the number of grid areas needed for stream size is complete. Record the mid-channel distance (m) from the station starting point to the downstream baseline for each grid area sampled. If obstructions prevent sampling of a grid area location, (i.e. impenetrable logjam) move upstream until grid baseline clears obstruction and record distance to baseline.

Table 2. Example of a random number datasheet for a stream less than 7 meters mean stream width using a 2.5 meter grid area. Random numbers are generated for starting location upstream from the station start and left or right bank location for placing grid areas. Diagram depicts quadrat locations within grid area and locations for taking depth and water velocity measurements.

Mussel Sampling Random Table - <7m MSW

1 (Enter number for random)

Stream_____ Station_____ Date_____

Due 8 Quadrats per Grid

Quad Location

D4	D5	D6		
V4	V5	V6		
21	22	23	24	25
16	17	18	19	20
11	12	13	14	15
6	7	8	9	10
1	2	3	4	5
D1	D2	D3		
V1	V2	V3		

Random Start 7 Then every 2.5m apart

Area	Riv Loc
1	2
2	
3	2
4	
5	2
6	
7	1
8	
9	2
10	
11	2
12	
13	2
14	

1=L Bank

2=R Bank

Proceed in order for R. loc.

(e.g. 1-2, 2-1)

Table 3. Sample of a random numbers table generated for sampling a stream less than 7 meters mean stream width. Eight random quadrat locations were selected for each of the 14 grid 2.5 x 2.5 m sampling areas.

	Area													
Quad	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	2	1	5	4	2	4	4	1	2	2	3	3	1	1
2	6	8	7	7	3	7	9	3	5	6	4	4	12	2
3	7	9	10	8	4	10	14	7	6	8	6	12	14	7
4	8	13	13	9	11	17	19	8	9	10	10	17	15	12
5	14	16	14	10	15	20	21	11	20	11	16	18	17	13
6	20	18	22	12	19	21	23	15	21	16	17	22	19	20
7	21	20	24	14	22	23	24	16	22	23	21	23	21	22
8	25	22	25	22	23	24	25	23	23	24	25	24	25	23

Table 4. Example of a random number datasheet for a stream between 7 and 14 m MSW using a 5 meter grid area. Random numbers are generated for starting location upstream from the station start and left or right bank locations for placing grid areas. Diagram depicts quadrat locations within grid area and locations for taking depth and water velocity measurements.

Mussel Sampling Random Table for Streams between 7-14m MSW

3 (Enter number for random)

Stream_____ Station_____ Date_____

Random Start (m) 3 Then every 10 m

Quadrat location within grid area

D4 V4				D5 V5				D6 V6	
91	92	93	94	95	96	97	98	99	100
81	82	83	84	85	86	87	88	89	90
71	72	73	74	75	76	77	78	79	80
61	62	63	64	65	66	67	68	69	70
51	52	53	54	55	56	57	58	59	60
41	42	43	44	45	46	47	48	49	50
31	32	33	34	35	36	37	38	39	40
21	22	23	24	25	26	27	28	29	30
11	12	13	14	15	16	17	18	19	20
1	2	3	4	5	6	7	8	9	10
D1 V1				D2 V2				D3 V3	

Area	Riv Loc
1	1
2	
3	1
4	
5	1
6	
7	2
8	
9	1
10	

1=L Bank

2=R Bank

Proceed in order for R. loc.

(e.g. 1-2, 2-1)

Table 5. Example of a random number datasheet for a stream between 15 and 24 m MSW using a 5 meter grid area. Random numbers are generated for starting location upstream from the station start and left, right or middle bank locations for placing grid areas. Diagram depicts quadrat locations within grid area and locations for taking depth and water velocity measurements.

Mussel Sampling Random Table for Streams between 15-24 m MSW

3 (Enter number for random)

Stream_____ Station_____ Date_____

Random Start (m) 0 Then every 10 m

Quad Location

D4 V4				D5 V5				D6 V6	
91	92	93	94	95	96	97	98	99	100
81	82	83	84	85	86	87	88	89	90
71	72	73	74	75	76	77	78	79	80
61	62	63	64	65	66	67	68	69	70
51	52	53	54	55	56	57	58	59	60
41	42	43	44	45	46	47	48	49	50
31	32	33	34	35	36	37	38	39	40
21	22	23	24	25	26	27	28	29	30
11	12	13	14	15	16	17	18	19	20
1	2	3	4	5	6	7	8	9	10
D1 V1				D2 V2				D3 V3	

Area	Riv Loc	
1	1	1=L Bank
2		2=R Bank
3		3=Middle
4	1	Proceed in order for R. loc.
5		(e.g. 3-1-2, 2-1-3)
6		
7	1	
8		
9		
10	2	
11		
12		
13	3	
14		
15		

Table 6. Example of a random number datasheet for streams greater than 25 m MSW using a 5 meter grid area. Random numbers are generated for starting location upstream from the station start and left, right or middle bank locations for grid area placement. Diagram depicts quadrat locations within grid area and locations for taking depth and water velocity measurements.

Mussel Sampling Random Table for Streams greater than 25m MSW

4 (Enter number for random)

Stream_____ Station_____ Date_____

Random Start (m) 7 Then every 10 m

Quad Location

D4 V4				D5 V5				D6 V6	
91	92	93	94	95	96	97	98	99	100
81	82	83	84	85	86	87	88	89	90
71	72	73	74	75	76	77	78	79	80
61	62	63	64	65	66	67	68	69	70
51	52	53	54	55	56	57	58	59	60
41	42	43	44	45	46	47	48	49	50
31	32	33	34	35	36	37	38	39	40
21	22	23	24	25	26	27	28	29	30
11	12	13	14	15	16	17	18	19	20
1	2	3	4	5	6	7	8	9	10
D1 V1				D2 V2				D3 V3	

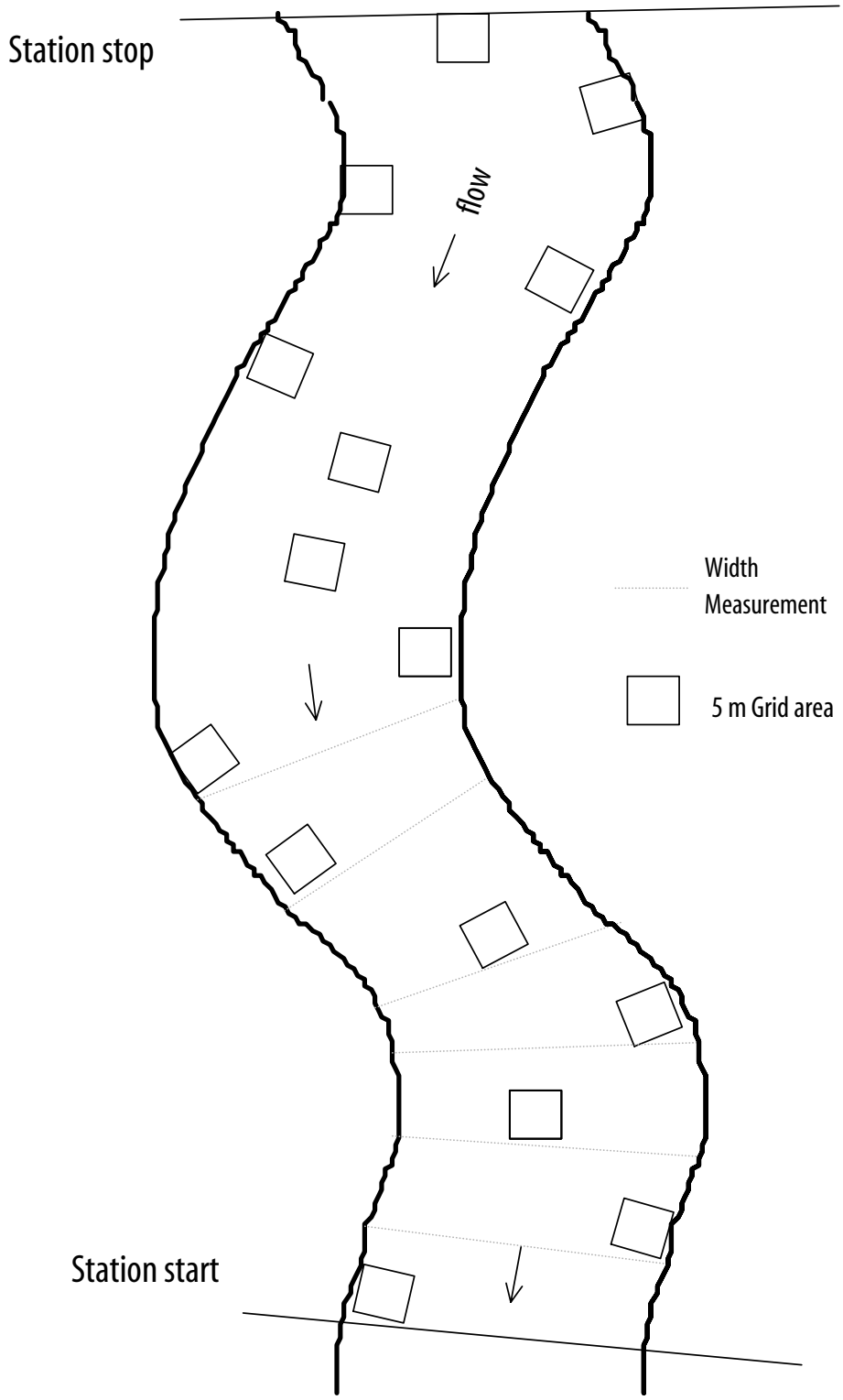
Area	Riv Loc
1	2
2	
3	
4	
5	2
6	
7	
8	
9	1
10	
11	
12	
13	2
14	
15	
16	
17	2
18	
19	
20	

1=L Bank
3=Middle Left
2=R Bank
4=Middle Right
Proceed in order for R. loc.
(e.g. 3-4-1-2, 2-3-4-1)

Table 7**.** Sample of a random numbers table generated for sampling streams greater than 7 m MSW. Twenty-five random quadrat locations were selected for each 5 x 5 m grid area.

	Area														
Quad	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	3	8	2	6	2	6	1	4	1	1	1	1	1	4	3
2	4	10	4	10	3	7	9	10	6	10	6	4	5	6	6
3	9	14	5	11	12	8	11	11	8	14	9	6	6	10	14
4	10	20	7	13	13	14	13	16	17	15	15	12	19	15	16
5	21	30	8	17	14	17	20	19	18	18	16	14	20	20	17
6	22	34	10	21	17	21	29	23	19	21	26	18	23	21	25
7	24	40	11	34	24	23	32	32	20	26	28	21	30	22	26
8	28	43	16	35	25	28	37	33	29	28	29	26	33	26	28
9	29	48	29	38	29	30	38	37	33	30	30	27	35	28	36
10	35	50	37	39	32	34	43	56	34	35	32	32	44	29	40
11	42	51	42	43	35	38	45	57	41	36	33	34	46	32	41
12	45	54	44	47	40	41	46	58	46	43	34	36	49	36	42
13	46	56	50	54	42	49	49	59	50	44	39	37	56	38	44
14	47	57	51	57	43	51	50	75	54	49	42	38	57	40	47
15	56	58	52	59	45	55	57	76	64	52	44	39	58	45	48
16	58	60	56	66	49	60	62	77	67	54	46	40	60	46	53
17	61	66	57	73	62	64	65	79	72	55	48	46	68	57	59
18	65	80	66	75	64	73	68	80	73	64	57	53	71	65	60
19	66	87	67	82	68	75	70	82	75	67	65	56	74	68	64
20	69	88	72	83	74	76	71	86	76	78	71	64	75	72	66
21	74	92	77	86	75	79	74	89	79	84	72	69	79	78	68
22	77	93	80	88	92	94	76	91	84	90	75	77	80	89	69
23	79	94	92	92	94	95	85	93	96	96	82	82	89	92	73
24	83	95	97	94	95	96	89	94	97	97	98	91	91	94	89
25	96	100	99	100	98	97	97	96	99	99	100	99	94	96	91

Figure 4. Diagram of random 5 m x 5 m grid area layout for a stream 17 m MSW. Habitat measurements are taken within each grid area and stream width measurements along a line perpendicular to the stream channel at each grid area. A total of 25 sub-samples are taken from each grid area using 0.25 m^2 quadrats.



Mussel Habitat

Mussel habitat will be collected within each grid area to establish mussel habitat associations within the stream channel. Mesohabitat is recorded for each grid site location. Depth and velocity measurements are taken at six points around the grid, at each corner and mid-point of the base and top grid ropes. Substrate types for detritus, clay, silt, sand, gravel, cobble/rubble, boulder and bedrock are recorded to the nearest 5%. Substrate types are visual or tactile estimates of substrate composition within the grid area. Mussels vary considerably in their habitat preferences; some species prefer swift flows with coarse rocky substrate, while others prefer shallow areas composed of silt-sand substrate. Vegetation coverage of macrophytes (emergent and submergent) and algae within the grid area are recorded to the nearest 5%. Macrophyte and algae coverage is visually estimated within the grid area. Abundant macrophyte or filamentous algae growth identifies streams polluted with excessive nutrients (usually nitrogen and phosphorus). Habitat measurements within grid areas will allow biologists to determine mussel-habitat associations that can then be used to estimate mussel populations for a site or stream reach based on available stream habitat within a site and stream reach.

Quantitative Sampling Procedure

Quantitative sampling should be conducted after an initial search is completed and station starting locations established. A two person team can be used in small streams (<7 m), and three person team or teams should be used in larger streams. The following procedure should be followed for quantitative sampling:

Complete mussel **Station** data sheet each time a site is sampled.

Establish a random starting distance and bank location for the first grid area to be sampled based on MSW established during the initial survey. Use grid random number data sheet established for MSW category.

Measure and record stream width at grid location, use a tape measure for small streams and laser range finder (if possible) for larger streams.

Stake out grid, using metal rebar rods being careful not to disturb area inside of grid. For bank grids, grid should be placed parallel to bank as close to the bank as possible (within 0.5 m) along the ordinary low water mark. Grid centerline should be parallel to flow. If emergent vegetation or obstructions prevent placement next to the bank, record the distance (m) from bank to inside grid line. Mid-channel grids should be located near the center for M, and at 1/3 and 2/3-channel widths from the left bank for M_1 and M_2 grids. Record the perpendicular distance from the Left bank to left grid line (looking upstream) for M, M_1 and M_2 grids.

Measure and record habitat data for grid area. Record grid habitat data on **Quadrat Search** datasheet. Record mesohabitat where grid is located. Depth (cm) and velocity (m/sec) are taken at three points on the downstream baseline. Velocity is measured at 0.6 water column depth. D_1 and V_1 are measured at point 0.25 m out from the downstream left corner of the grid. D_2 and V_2 are measured at the mid-point on the downstream baseline of the grid. D_3

and V_3 are measured at point 0.25 m in from the downstream right corner of the grid. $D_4$and V_4 are measured at point 0.25 m out from the upstream left corner of the grid. D_5 and V_5 are measured at the mid-point on the upstream baseline of the grid. D_6 and V_6 are measured at point 0.25 m in from the upstream right corner of the grid. Measure and record substrate percentages, macrophyte and algae coverage. In turbid waters, substrate composition may have to be estimated by feeling substrate. Once habitat data is collected, grid area may be searched for mussels using quadrats.

Select column of random numbers for quadrat locations within grid area from random numbers table datasheet. Begin with the lowest number and proceed with the next highest random number. Quadrat should be carefully placed at the selected number location, using grid ropes and colored twine tuffs as a guide for placement, once quadrat is placed, visually search the quadrat for mussels and place all mussels found in a mesh bag. In turbid waters, use a light tactile sweep across the quadrat to locate mussels. Next, lightly fan the quadrat to remove fine substrates and reveal buried mussels. Be careful not to fan too hard as to wash away small mussels. Lastly, moderately fan the substrate to a depth of 5 cm to expose deeply buried mussels. In heavy macrophytes, the plant stems and leaves can be cut away after the first visual or tactile search is finished. Do not pull plants out by the roots, as this may dislodge and wash away mussels. All mussels found inside quadrat area should be placed in a mesh bag numbered with the corresponding quadrat location. Numbered bags can then be handed to non-searching personnel for identification and measurements. Searcher then proceeds to next random location and repeat procedures.

All mussels (live and dead) are identified to species and counted for each quadrat searched and recorded on **Quadrat Search** datasheet. Be careful to record correct quadrat number and quadrat location within grid for each quadrat. All live mussels collected within each quadrat are identified, measured for length, aged, sexed and coded for shell wear on **Mussel Length Age** datasheet. Record all species found using scientific name (e.g. P. grandis), including dead shells. If no mussels are found, then species found is recorded as **No mussels**. All live mussels should be handled gently and re-bedded immediately back to the location where they were found once identified and measurements taken. Mussels not identified should be clearly photographed or a voucher specimen brought back to lab for positive identification by a malacologist familiar with regional fauna. Uncertain identities should be photographed and experts consulted or dead shell retained. Mussels should be measured for length along the longest line parallel to the hinge line. Mussels should be externally sexed if possible for a species (e.g. *Epioblasma triquetra*, *Lampsilis siliquoidea*). Count all mussels 3 years old or less by species. Mussels are externally aged to the last growth ring using the left valve if possible, do not count the edge as the last growth ring during the growing season. Age the first 100 mussels of each species, if mussel cannot be aged, record age as NA. Record shell wear for the first 100 mussels aged for each species. Individual shell wear is coded from 0-4 based on overall shell condition where:

0 = no wear on shell surface, slight wear may be present on beak sculpture.
1 = 0-25% of surface worn, light wear.
2 = 25-50% of shell surface worn, light to moderate wear some pitting.
3 = 50-75% of shell surface worn, some deep pitting.
4 = 75-100% of shell surface worn, deep pitting, badly eroded surface.

When all quadrats have been sampled and mussels replaced within grid area, proceed to the next grid location in sequence and repeat sampling procedure. Continue sampling procedure until the required number of grid areas have been sampled.

End survey.

Mussel Identification

All mussels collected should be identified to species. Refer to Table 8 for a list of the scientific name, common name, and status of mussels found in Wisconsin. Voucher 1-2 individuals (use dead shells when possible), or take a high quality digital photograph of any mussels that need to be positively identified. A malacologist familiar with the identification of regional unionid mussel fauna should be consulted to positively identify all mussels collected while sampling. Identification of freshwater mussels can be difficult for inexperienced collectors due to the subjective nature of identifying shell characters and the great variability in size, shape and color of many species. The following literature can be referenced to help identify freshwater mussels found in Wisconsin waters.

Cummings, K. S., and C. A. Mayer. 1992. Field guide to freshwater mussels of the Midwest. Illinois Natural History Survey Manual 5. 194 pp.

Mathiak, Harold A. 1979. A river survey of the unionid mussels of Wisconsin 1973-1977. Sand Shell Press, P.O. Box 44 Horicon, WI 53032.

Minnesota Department of Natural Resources. 2003. Field guide to freshwater mussels of Minnesota. 144 pp. Minnesota Department of Natural Resources, St. Paul.

Stern, E. M. 1990. An illustrated key to the freshwater mussels (Bivalvia: Unionidae) of Wisconsin. Reports of the Museum of Natural History 20. University of Wisconsin at Stevens Point. 75 pp.

Wisconsin Department of Natural Resources. 1985. Freshwater mussels of the Upper Mississippi River. 63 pp. Wisconsin Department of Natural Resources, Madison.

Additional information on identification and ecology of freshwater mussels can be found on Internet Web resources. The following links provide a good starting point to obtain more freshwater mussel information:

Freshwater Mollusk Conservation Society, http://ellipse.inhs.uiuc.edu/FMCS/

American Malacological Society, http://data.acnatsci.org/ams/

Scientific and common names of all mussels collected should follow nomenclature reported in Turgeon 1998. Use scientific name to avoid confusion of mussel species, many species have been given several common names which may vary between collectors and locality.

Table 8. Wisconsin mussel species list. Scientific, common name and current status (2004). For status: LE=federal endangered, SE=state endangered, ST=state threatened, SC= state special concern, EX=extirpated from state.

SCIENTIFIC	COMMMON		SCIENTIFIC	COMMMON	
Actinonaias ligamentina	Mucket		*Obovaria olivaria*	Hickorynut	
Alasmidonta marginata	Elktoe	SC	*Plethobasus cyphyus*	Sheepnose (Bullhead)	SE
Alasmidonta viridis	Slippershell	ST	*Pleurobema sintoxia*	Round Pigtoe	SC
Amblema plicata	Threeridge		*Potamilus alatus*	Pink Heelsplitter	
Anodonta suborbiculata	Flat Floater	SC	*Potamilus capax*	Fat pocketbook	EX
Anodontoides ferussacianus	Cylindrical Papershell		*Potamilus ohiensis*	Pink Papershell	
Arcidens confragosus	Rock Pocketbook	ST	*Pyganodon cataracta*	Eastern Floater	SC
Cumberlandia monodonta	Spectaclecase	SE	*Pyganodon grandis*	Giant Floater	
Cyclonaias tuberculata	Purple Wartyback	SE	*Quadrula fragosa*	Winged Mapleleaf	LE/SE
Ellipsaria lineolata	Butterfly	SE	*Quadrula metanevra*	Monkeyface	ST
Elliptio complanata	Eastern Elliptio	SC	*Quadrula nodulata*	Wartyback	ST
Elliptio crassidens	Elephant-Ear	SE	*Quadrula pustulosa*	Pimpleback	
Elliptio dilatata	Spike		*Quadrula quadrula*	Mapleleaf	
Epioblasma triquetra	Snuffbox	SE	*Simpsonaias ambigua*	Salamander Mussel	ST
Fusconaia ebena	Ebonyshell	SE	*Toxolasma parvus*	Lilliput	
Fusconaia flava	Wabash Pigtoe		*Tritogonia verrucosa*	Pistolgrip (Buckhorn)	ST
Lampsilis cardium	Plain Pocketbook		*Truncilla donaciformis*	Fawnsfoot	SC
Lampsilis higginsi	Higgins Eye	LE/SE	*Truncilla truncata*	Deertoe	
Lampsilis siliquoidea	Fatmucket		*Utterbackia imbecilis*	Alewife Floater	
Lampsilis teres (anodontoides)	Yellow Sandshell	SE	*Venustaconcha ellipsiformis*	Ellipse	ST
Lampsilis teres (teres)	Slough Sandshell	SE	*Villosa iris*	Rainbow	SE
Lasmigona complanata	White Heelsplitter				
Lasmigona compressa	Creek Heelsplitter		Fingernail Clams		
Lasmigona costata	Fluted-Shell		*Pisidium cruciatum*	Ornamated Peaclam	SC
Leptodea fragilis	Fragile Papershell				
Leptodea leptodon	Scaleshell	EX	Exotic clams		
Ligumia recta	Black Sandshell		*Corbicula fuminea*	Asiatic clam	
Megalonaias nervosa	Washboard	SC	*Dreisenna polymorpha*	Zebra mussel	
Obliquaria reflexa	Threehorn Wartyback				

Turgeon, D. D., J. F. Quinn, A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione and J. D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks, 2nd edition. American Fisheries Society, Special Publication 26, Bethesda, Maryland.

Mussel Survey Reporting

Reporting summaries of mussel sampling is mandatory for each survey or project. Copies of the mussel summary report are to be filed with the following WDNR personnel as appropriate per project:

WDNR project coordinator overseeing the project - Regional.

WDNR Bureau of Endangered Resources – Madison.

WDNR Mussel Atlas coordinator – Madison.

Summary reports are to be filed in a timely manner following the completion of the survey or project. Reports should contain a brief write-up that includes the following:

Person or persons doing survey
Person or agency requesting survey
Reason for conducting survey
Project permit number if applicable
General site description
Methods
Station datasheet
Map of site or sites
Summary Results for each sampling method (Quantitative or Qualitative) conducted
Any problems encountered during survey that may effect results
Assessment of potential impacts to the mussel community

Mussel Sampling Data Sheet - Station (*indicates required field) p. __ of __

Waterbody ID _____

*Stream: _____ Station: _____ Site Mile _____

*Date (mm/dd/yyy): ____ / ____ / _____ *Collectors: _____

*County: _____ *Station Start GPS Location: ___ _____ . _____
(hddd.ddddd)

*T _____ R _____ Sec _____ QQ_____ Q _____ _____ . _____

Nearest Road/Access: _____ Dist. from access Above _____ Below _____

*Time (24 hr): _____ *Water Level: ____ Normal Above _____ (0.1 m) Below _____ (0.1 m)

*Air Temp (C°): _____ *Water Temp (C°): _____ Conductivity (umhos): _____ Turbidity (NTU): _____

*Water Clarity: Cl St Slt Turb *Visibility (cm): _____ Measure depth to which fine substrate can be clearly seen

*Gradient (flow): N L M H *Natural ___ Tailwater (< 5.0 mi below dam) ___ Dist below dam _____

*SAMPLING STRATEGY: _____ (Initial – Timed Search – Quadrats)

*Search Time (24 hr): Start _____ Stop _____ Total (min) _____ *Bank: L R B

*Area searched Length (m) _____ Mean Width (m) _____

*Mussels present: Y N *Distance to live mussels (m) _____

*General Habitat Description

River Widths (Approx. every 2 stream width apart) _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

Macro Habitat Length (m)	Substrate (nearest 5%)	Instream Vegetation (nearest 5%)	Riparian Land Use (Nearest 5% within 5 m)	Artificial Bank Features Length (m)
Pool _____	Detritus _____	Emergent _____	Wetland _____	RipRap _____
Run _____	Clay _____	Submergent _____	Meadow _____	Seawall _____
Riffle _____	Silt _____	Algae _____	Woodland _____	Other _____
Rapids _____	Sand _____		Pasture _____	List Other _____
Other _____	Gravel _____		Cropland _____	
List Other _____	Cobble _____		Developed _____	
	Boulder _____		Other _____	
	Bedrock _____		List Other _____	
	Other _____ List Other _____			

Mussel Sampling Data Sheet – **Timed Search** (*indicates required field) p. __ of __
(Minimum 4 man/hr on streams < 15 m and 8 man/hr on stream > 15 m)

Waterbody ID _______________

*Stream: ____ Station: ____________________ Site Mile __________

*Date (mm/dd/yyy): ____ / ____ / _______ *Collectors: ____

*Search Time (24 hr): Start ________ Stop ________ Total (min) ________ *Bank: L R B

*Number of searchers _____ *Area searched Length (m) _____ *Mean Width (m) _____

*Species Found	*Number Live	*Number Live ≤ 3 yr	Dead

Mussel Sampling Data Sheet – **Quadrat Search** (* indicates required field) p.___ of ____

*River/Stream____________________ *Station_____________ *Date_______________ * Collectors __________________

GPS Loc ____ ______ __________
_______ __________ *Start Time ________ Stop Time _________ Total __________

*Area # _____ Start Dist_____ *River Width (m) _____ *Bank L R M __ *Dist from Bank (m) ______ (L bank for middle)

*Mesohabitat:__________________ *Substrate (5%) 1 Det_____ 2 Cl_____ 3 Slt_____ 4 Snd_____

*Depth (cm) D1_____ D2_____ D3_____ D4_____ D5_____ D6_____ 5 Grv_____ 6 C/R_____ 7 Bld_____ 8 BR_____

*Velocity (m/s) V1_____ V2_____ V3_____ V4_____ V5_____ V6_____ *Mac (5%) _____ *Algae (5%) _____

Q#	Q Loc	Species	Live	Dead	Q#	Q Loc	Species	Live	Dead

Mussel Sampling Data Sheet – **Length – Age** - Sex - Condition (* indicates required field) p.___ of ____

*River/Stream____________________ *Station______________ *Date_______________ *Collectors __________________
____

Length (mm) Cond (shell wear) **0** = No wear **1** = 0-25% **2** = 25-50%
Age (visible growth rings excluding edge) **3** = 50-75% **4** = 75-100%

Area	Quad	Species	Length	Age	Sex	Cond

Area	Quad	Species	Length	Age	Sex	Cond

Mussel Relocation Protocol

Disturbance to mussels is sometimes be unavoidable (i.e. new bridges, boat piers, instream structures), if impacts are unavoidable, mussels within the impact area may be removed and relocated to safe habitat nearby. This protocol is intended as a framework for the development of a Project Specific Relocation Plan.

Relocation Schedule

Relocation will occur in a timeframe that allows completion of all mussel relocation work immediately prior to initiation of activities that impact the streambed. This is to ensure that mussels do not emigrate or recruit in the relocation area before construction is initiated. Time restrictions may also apply based on the gravid period of special status species. To minimize thermal stress to the mussels, water temperature should be at least 40^{o}F.

Relocation Personnel

A malacologist experienced in unionid collection and monitoring techniques will coordinate all relocation and monitoring efforts. The malacologist must be familiar with the identification of unionid mussel fauna and have particular expertise in the identification of rare species. If deemed necessary, a quality assurance (QA) team may be designated to ensure project quality.

Relocation Team

The relocation team is responsible for relocation and monitoring efforts. The team provides personnel and equipment needed to complete relocation and monitoring according to this protocol. The team is responsible for acquiring all state, federal or other permits necessary for handling mussels at the site. At a minimum, the team consists of an experienced malacologist and experienced field staff. Additional technical support will be used as needed. Sufficient staff must be available to ensure safety and quality of work and minimize stress to the mussels during all phases of the relocation effort. If a QA team is designated, the relocation team will coordinate with them throughout the relocation and monitoring effort. The relocation team will prepare reports following both the relocation and monitoring field efforts.

Quality Assurance Team

If designated, the QA team, minimally, consists of an experienced malacologist certified to dive. Additional field staff may be needed periodically during relocation efforts. The QA team is responsible for ensuring that all relocation and monitoring protocols are followed and require any corrective actions of the relocation team. The QA team is also responsible for supervising and assisting monitoring design and setup, and accuracy in data recording and species identification. Summary reports evaluating the relocation will be prepared following the relocation effort and last monitoring effort.

The QA team will observe collection, handling, species identification, placement procedures, and monitor air and water temperatures during the relocation effort. The QA team will check collection areas to ensure all (100%) or a designated acceptable percentage (e.g. 95%) of mussels are collected

from impact areas. The QA team will inspect the transplant area to ensure that mussels are distributed properly throughout the transplant site and positioned correctly in the substrate.

Transplant Area

Transplant Area Location

Prior to the relocation effort, the relocation team will select a suitable transplant site. The site must be approved by the QA team or natural resource agency staff and should:

1. Be close to the collection area.
2. Have similar or better water quality, substrate, and fish fauna to the collection site.
3. Not be influenced by factors detrimental to unionids (e.g. point discharge, dredging, navigation).
4. Have an existing or historical mussel population with similar species composition.

Transplant Area Delineation

Randomly placed quadrat samples will be collected to an appropriate depth within the transplant site to determine existing unionid density and substrate composition in the transplant site before relocating unionids. Mussels collected in quadrats would be returned to the transplant area.

Unionid Collection from the Impact Area

Bridge Site/Impact Area Delineation

Mussels will be relocated from all areas of designated impact. If the impact is a bridge, the new bridge structure and any temporary structures used to construct the bridge (i.e. causeways, temporary bridge, barging areas) should be considered in the area of impact. All impact areas should include an appropriate buffer (i.e. 1 to 2 m for areas of direct impact). Delineated areas should be clearly marked. The markers will remain in place throughout the relocation effort, AND all instream construction activities. This is to ensure that impacts to the streambed are contained within the areas where mussels were removed. The appropriate state or federal natural resource agency should review any changes in construction plans and modify the impact area and estimates of mussels prior to any field work.

As an example - A bridge relocation might entail removing mussels from areas around the permanent bridge piers and temporary causeway used to build the bridge. Estimates of area of impact could be determined from the bridge plans (including a buffer) for the piers and causeway. The estimate of mussels impacted would be based on original survey results from the site. Areas with substrate unsuitable for mussels would not need to be included in the delineated area of impact.

Unionid Collection from the Bridge Site/Impact Area

Following delineation, the relocation team will establish transects within the collection area. All mussels in the area will be collected. Collecting from downstream to upstream is recommended as the most efficient approach. All mussels will be collected into mesh bags that will be

maintained in the water. The number of mussels collected in each bag will be based on the size and number of mussels and adjusted to avoid overcrowding. All areas will be traversed at least twice to ensure all or an acceptable percentage of the total number (i.e. 95%) of mussels within the area are collected.

Inspection of Bridge Site/ Impact Area

Areas will be inspected for collection thoroughness, this will be accomplished by the QA team if one is designated. Searches will be conducted within the delineated areas and results will be compared with total collection density. If results indicate an unacceptable percentage of the estimated number remains, the relocation team will recollect the area until an acceptable removal percentage has been achieved.

Holding, Processing & Transport

Following collection of mussels from the substrate, the mussels will be maintained in water at ambient temperature during holding and subsequent transport to the relocation site. Air and water temperatures should be monitored throughout the relocation process. All unionids will be marked to distinguish them from resident unionids at the relocation site. At a minimum, all mussels collected will be identified, counted, marked, and relocated. Special status species will be identified, sexed (if appropriate), measured, aged, and uniquely marked on both valves. Gravidity of females should be noted if conducted cautiously. The method to be used for marking mussels should limit handling, be accomplished with a minimum out of water time, be fairly permanent, match shell type and size, and be easily readable after several years.

All mussels will be carefully handled and, except for processing time, will be held in water at ambient temperatures (e.g. bags in river, flow-through tank, etc.) while out of the substrate. Time out of substrate will be limited to the time required for collection, processing, holding and transport between the collection and relocation areas. Time out of substrate will not exceed a predetermined amount of time, four hours is recommended as a maximum.

Placement

All special status species will be relocated to an area established at the transplant site for monitoring rare species. The special status mussels will be hand-placed in a natural position within the substrate such that density following transplant does not exceed twice the initial density of the relocation site. The remaining mussels may be distributed by hand along the surface of the substrate or hand-placed in the substrate. Mussels should be distributed evenly (by boat, diver, or wading) in suitable habitat. The transplant area will be inspected, following relocation, to ensure unionids are distributed properly.

Monitoring

A monitoring program is essential to evaluate the success of any relocation project. At a minimum, the monitoring program should include a quantifiable assessment of special status species survival. Survival will be assessed by placing special status species in a known area and monitoring their condition at one month and at least one year or yearly intervals following relocation. Collected mussels will be identified, counted, measured, aged, and replaced. Special status species collected

during the monitoring effort will be returned to the location where they were collected. At a minimum, mortality of relocated special status species will be compared with mortality of other relocated and resident mussels.

Monitoring Personnel

Personnel from the relocation team will be responsible for conducting all monitoring. A designated subsample or the entire relocation site will be searched by quadrats or other appropriate quantifiable method (e.g. transects along a grid). The area or number of quadrats should be sufficient to assess at least half of the species of concern and should address survival of other relocated species if possible. If designated, a member of the QA team will be on hand to ensure animals are handled properly, all checks are performed properly, and assist with sample collection and other fieldwork as needed.

Initial and One-Month Monitoring

Mortality should be assessed upon completion of the initial relocation effort. To assess immediate mortality, a one month swim-over may be conducted over the area being monitored and all fresh-dead mussels retrieved. In addition, a cursory search outside of the relocation area should be conducted to recover shells that may have been displaced and observe mussels that may have moved. General condition and behavior of the relocated and resident mussels should be noted.

Yearly Monitoring

During yearly monitoring a swim-over survey should be conducted over the entire relocation area being monitored and all fresh-dead shells, marked and unmarked, retrieved. A subsample or the entire relocation site will be searched by either quadrats or other appropriate quantifiable method (e.g. transects along a grid) and all live mussels encountered will be identified and marks noted if present. Marked special status species are to be measured and checked for gravidity, if appropriate and not stressful. All mussels are to be handled underwater or with a minimum of out of water time. The location of all special status mussels encountered within the relocation area will be noted. Special status species will be replaced in the substrate in the same location from which they were collected and all other mussels will be replaced in the same general location.

Possible Further Monitoring

If data after one year of monitoring indicates survival has not stabilized or is less than an acceptable limit (e.g. 90%, 75%.), the relocation team will coordinate with the QA team (if designated) and appropriate state or federal agencies to determine if monitoring should be continued.

Reports

Relocation Team

A report will be prepared following completion of the relocation, detailing methods, and any problems with or suggestions to improve the relocation process, data from relocated mussels, population characteristics in the relocation area before and after relocation, and data from the impact

site. Monitoring reports following each effort will detail methods and results, and any problems encountered.

Quality Assurance Team

Summary reports will be prepared by the QA team following completion of the relocation effort and final monitoring effort. The relocation report will include an overall assessment and analysis of methods, problems encountered and corrective actions. Following final monitoring, a comprehensive summary report will be prepared. The final report will include an evaluation of relocation and monitoring methods, results, a discussion of techniques and results based on the relocation team report, and recommendations for future relocation efforts.

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